THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXVI MAY, 1950 NUMBER 3

A DISTINCTIVE VACUOLAR NEPHROPATHY ASSOCIATED WITH INTESTINAL DISEASE*

J. P. KULKA, M.D.,† C. M. PEARSON, M.D., and S. L. ROBBINS, M.D.

(From the Department of Pathology and Bacteriology, Massachusetts General Hospital, and the Mallory Institute of Pathology, Boston City Hospital, Boston, Mass.)

Small hydropic vacuoles are frequently found in the epithelial cells of the proximal nephric tubules in many different disease states. These vacuoles are generally multiple, and do not displace the nucleus or distort the cell contours. On the contrary, it is rare to find giant and usually solitary vacuoles not containing either fat or glycogen which reach such a size that they displace the nuclei basally and balloon out the cells toward the tubular lumen. Except for a few isolated reports, such a microscopic picture has been described previously in only two conditions, bacillary dysentery[1,2] and poisoning with diethylene glycol or dioxane.[3]

During the routine examination of post-mortem material we encountered several examples of this renal lesion in cases with widely differing gastro-intestinal disorders in which neither bacillary dysentery nor glycol poisoning was present. This chance discovery led to a review of approximately 150 cases of various types of intestinal disease including 72 cases of ulcerative colitis. A distinctive renal vacuolation was present in 15 instances which was apparently identical to that associated with bacillary dysentery. Two cases have been published previously as clinicopathologic conferences.[4,5] The bowel disturbance in these cases was at times obstructive rather than diarrheal and in one instance the intestinal lesions were minimal and asymptomatic. In all cases, however, the primary disease was finally overshadowed by systemic disturbances with at least some degree of electrolyte imbalance, malnutrition, anemia, and probably sepsis.

The present study concerns itself with an analysis of the clinical and anatomical features common to these 15 cases. An effort is made to

* Received for publication, May 7, 1949.

† Now at Department of Pathology, Harvard Medical School, Boston, Mass.

define this pathologic entity more clearly and to evaluate possible pathogenic factors.

Clinical and Laboratory Findings

Tables I and II summarize the pertinent clinical and laboratory

TABLE I

Clinical Findings

Case no.	Age	Sex	Principal diseases			
			Intestinal	Other	Diarrhea	Other excessive fluid loss
1 M.G.H. 10567	27 yrs.	M	Chronic ulcerative colitis	Peritonitis; fecal; perirectal abscess	Intermittent, 8 years; acute, 3 weeks	Miller-Abbott tube
2 B.C.H. A-42-299	19 yrs.	F	Chronic ulcerative colitis	Peritonitis; fibrinous; bronchopneumonia	10 weeks	
3 B.C.H. A-35-202	50 yrs.	M	Chronic ulcerative colitis	Peritonitis; purulent; bronchopneumonia	5 months	Vomiting; ileostomy
4 B.C.H. A-46-12	44 yrs.	M	Acute ulcerative colitis	Bronchopneumonia, uremia	4 weeks	Vomiting
5 B.C.H. A-46-619	35 yrs.	F	Chronic ulcerative colitis	Peritonitis; fibrinopurulent; rectovaginal fistula	3 months	
6 B.C.H. A-40-828	44 yrs.	F	Chronic ulcerative colitis	Peritonitis; fibrinous pericarditis; rectovaginal fistula	13 months	Ileostomy
7 B.C.H. A-46-513	53 yrs.	M	Chronic ulcerative colitis	Chronic hemolytic anemia; uremia; bronchopneumonia	11 months	
8 M.G.H. 11423	20 yrs.	F	Chronic ulcerative colitis	Miliary tuberculosis	12 months	Ileostomy
9 M.G.H. 10559	24 yrs.	F	Regional enteritis	Tetany	Intermittent, 5 years; acute, 5 months	Ileostomy
10 M.G.H. 11340	46 yrs.	F	Subacute duodenal obstruction	Peritonitis; uremia; tetany	4 weeks	Nelson suction; vomiting
11 B.C.H. A-47-93	72 yrs.	M	Diverticulitis	Arteriosclerotic heart disease; bronchopneumonia; uremia	2 weeks	Vomiting
12 B.C.H. A-47-388	55 yrs.	F	Carcinoma of ascending colon; acute ulcerative ileocolitis	Peritonitis, fecal	None	Miller-Abbott tube
13 M.G.H. 8556	40 yrs.	F	Ileocolic fistula; ulcerative sigmoidoproctitis	Hodgkin's lymphoma; peritonitis; hydronephrosis; uremia	1 day	
14 B.C.H. A-47-475	7 mos.	M	Acute enteritis	Abscess of leg; otitis media; bronchopneumonia	2 weeks	Vomiting
15 M.G.H. 6728	64 yrs.	F	Carcinomatous infiltration of bowel serosa, subclinical	Carcinomatosis; subacute cholangitis; pyorrhea	None	

findings. The most striking common feature was the presence of intestinal disease of 4 or more weeks' duration in all 15 cases. Eight of these patients had typical nonspecific ulcerative colitis. The remaining 7 had intestinal lesions of widely different types which, in one instance,

Signs and Symptoms				Therapy		
Intestinal by-pass	Intestinal obstruction	Nutritional status	Peripheral edema	Blood administered	Total fluid intake; terminal, 7 days	Possible nephrotoxins
Ileotransverse colostomy	+	Poor	0	500 cc.	28,300 cc.	Sulfasuxidine
None	0	Fair	++	4000 cc. in 3 weeks	18,000 cc.	Sulfathiazole; sulfaguanidine
Ileostomy	0	Poor	0	2000 cc. in 2 weeks	19,800 cc.	
None	0	Poor	0	None	3000 to 4500 cc. per day	Sulfadiazine
None	0	Very poor	++++	"Many" times	25,800 cc.	Sulfasuxidine
Ileostomy	0	Fair	+++	7000 cc. in 10 weeks	33,700 cc.	Sulfadiazine (with reaction)
None	0	Fair	+	2000 cc. (with reaction)	2000 to 6000 cc. per day	Sulfadiazine; carboxysulfathiazole
Ileostomy	0	Poor	++	2500 cc.	18,300 cc.	
Ileostomy	+	Very poor	++	4800 cc. in 10 weeks	27,500 cc.	
Multiple small bowel anastomoses	+	Very poor	+	2500 cc.	13,500 cc.	
None	0	Fair	+	None	17,000 cc.	Mercupurin
None	+	Fair	+++	"Several" times	Not available	Mercupurin
Ileocolic fistula	+	Poor	0	500 cc.	32,200 cc.	
None	0	Poor	+	None	6,000 cc.	Sulfadiazine
None	0	Fair	+	None	18,600 cc.	

consisted only of minimal carcinomatous seeding of the serosa of the bowel with no resulting clinical manifestations.

Diarrhea was the most common and usually the chief symptom. In 12 patients it lasted in more or less acute form for 2 or more weeks, but in one it began only 1 day before death and in 2 it was absent altogether. Seven patients had varying degrees of bowel short-circuit, either as a result of disease or of surgical procedures. Vomiting was present in 5, and in 2 of these it was the presenting symptom. Subacute or chronic intestinal obstruction was observed in 5 patients and in most of these alternating diarrhea and constipation were present, although at times in mild form. The level of obstruction ranged from the terminal duodenal region to the rectosigmoid. Paralytic obstruction may have existed terminally in some of the cases but the occasional existence of a terminal "shock-like" state was the only indication for such a supposition.

All cases had some degree of sepsis and fever but the temperature was rarely elevated over 103° F. and the pulse rate was usually only moderately increased. In addition to the inflammatory bowel lesions in 14 of the 15 cases, there were 8 instances of acute peritonitis, 6 of bronchopneumonia, 3 of perirectal abscesses or fistulae, 1 of miliary tuberculosis and 1 of subacute cholangitis.

The nutritional status of all patients was impaired, in accordance with the presence of severe intestinal disease or malignancy. The patient designated as case 15, although described as well nourished, had some hypoproteinemia and anemia. The severity of the malnutrition bore no relation to that of the renal lesions.

Some degree of anemia was present in all but case 4, and in that instance dehydration and hemoconcentration may have distorted the blood picture. Values as low as 3.2 gm. of hemoglobin per 100 cc. were obtained in case 7, which was complicated by a severe hemolytic process, but in general the hemoglobin levels ranged from 9 to 11 gm. per 100 cc.

Hypoproteinemia of from 3.1 to 5.0 gm. per 100 cc. was present at one time or another in at least 10 of the patients. It was not determined in 3 others, and in the 2 remaining cases the serum protein levels were normal, possibly as a result of hemoconcentration. The albumin-globulin ratio was determined in 4 instances and in 3 of these there was a slight reversal. Peripheral edema was present to some degree in 11 cases and was generally proportional to the decrease in serum proteins. In contrast, there was no definite correlation between edema and the amount of fluids given or the severity of the renal vacuolation.

The fluid and electrolyte balance was severely deranged in most of the cases and was abnormal in all. Usually there was a marked fluid

Laboratory Findings *

Case no.	Blood							Urine			
	Hemoglobin (gm./100 cc.)	Protein (gm./100 cc.)	Albumin-globulin ratio	Serum chlorides (mEq./l.)	Serum non-protein nitrogen (mg./100 cc.)	CO_2 vols. %	Miscellaneous	Minimum output (per day)	Specific gravity	Maximal albuminuria	Miscellaneous
1	10.5	6.2 to 4.7		82.9	36 to 45			800	1.012	++++	15 r.b.c. and 1–2 w.b.c./h.p.f.
2	12.4	4.08 to 5.4		99.0	18			"Normal"	1.006	+	
3	9.4			80.0	25			900	1.010 to 1.025	++	Granular and hyaline casts, few r.b.c. and w.b.c.
4	15.0	6.07		87.0	91						
5	7.9	3.1	1.5/1.6	88.0		39 and 68		800	1.004 to 1.010	+	
6	8.0	4.2	2.0/2.2	85.0	15 to 22	53 and 69	Sodium, 132 mEq/l.	800	1.002 to 1.020	+	
7	7.8 and 3.2				41 to 70		Icteric index, 50	200	1.003 to 1.014	+	Granular casts
8	8.6	4.88	2.8/2.1	89.0 and 99.0	23		Van den Bergh: direct 1.1; indirect, 1.8	250	1.004 to 1.025	0	
9	10.0	4.7	2.3/2.4	98.2 and 73.6	20	31	Calcium, 9.7 mg.; phosphorus, 2.0 mg.	500	1.010 to 1.012	++	Trace of calcium
10	12.0	5.9, 4.3, and 6.0		65.0 and 77.0	16 to 82	41 and 18		500	1.010	++	20–50 w.b.c./h.p.f.
11	12.0	7.2 and 4.5		92.0 and 80.0	36 to 185	36 and 23		Terminal oliguria	1.012 to 1.018	±	
12	Not reported Hematocrit, 36	4.9		96.0	27			Not known	1.001 to 1.016	+	
13	Not reported Hematocrit, 18 Red blood cells, 1,500,000	3.7		97.0	150 to 210	20 and 46	Phosphorus, 10.3 mg.	500	1.014	++++	Few r.b.c. and w.b.c.
14	Red blood cells, 1.6 to 5.1 millions										
15	10.9	5.6		100.0	32		Calcium, 9.4 mg.; phosphorus, 3.96 mg.; alkaline phosphatase, 10.2 units	900	1.006 to 1.022	+	Bile

* These findings all pertain to the patients' final admissions. Where several values are given, they are presented in chronologic order except in the case of the urine specific gravities where the minimum and maximum values are indicated.

loss from the intestinal tract. Thus in case 9 up to 3100 cc. drained from an ileostomy in 24 hours, and in several instances of severe diarrhea the loss may have been even greater. All patients received considerable quantities of parenteral fluids. Although occasional transfusions of whole blood or plasma were given, usually the fluids consisted of equal proportions of 5 per cent glucose in distilled water and 5 per cent glucose in normal saline solution, or normal saline alone. The daily amount of fluid administered differed widely and ranged in one instance up to 6000 cc. Because of the difficulty in determining the exact amounts of the losses incurred, there was probably both overtreatment and undertreatment as manifested clinically by the frequent presence of edema or dehydration.

The serum chlorides were decreased below 96 milli-equivalents per liter in 9 patients. In 8 of these there were ileostomies, vomiting, or other sources of fluid loss from the upper intestinal tract. The lowest level, that of 65 mEq./l., was reached in case 10 as a result of high intestinal obstruction, constant gastric suction, and vomiting. In case 5, the chlorides dropped to 88 mEq./l., although there was no bowel short-circuit and the only source of fluid loss was severe diarrhea. There was no correlation between the degree of hypochloremia and the severity of diarrhea or of the renal vacuolation.

Although marked loss of basic ions must also have occurred in most, if not all cases, the serum levels of sodium, calcium, and phosphorus were not decreased in the few instances in which determinations were made. The serum phosphorus of 10.3 mg. per 100 cc. in case 13 was probably the result of hydronephrosis.

The CO_2-combining power varied greatly and even in the course of an individual case there was frequently an apparent shift from acidosis to alkalosis and vice versa. However, since no determinations of serum bicarbonate were made, no definite conclusions as to the pH of the blood can be reached. For the same reason the tetany in cases 9 and 10 may be ascribed to alkalosis, despite the apparently inconsistent values for the CO_2-combining power.

The clinical manifestations of renal dysfunction were varied and correlated poorly with the severity of vacuolation. The most common urinary finding was albuminuria, which was present to some extent in all but one case in which a determination was made. In only 2 cases, however, was albuminuria marked: in case 1 where it coincided with the most severe hydropic nephrosis, and in case 13 where hydronephrosis was present as a complicating factor. In the other cases albuminuria was slight to moderate and in 5 of them it appeared only terminally.

The nonprotein nitrogen rose above normal in 6 cases. The highest level of 210 mg. per 100 cc. was reached in case 13 and was probably mainly the result of hydronephrosis. Five of the other cases, with no complicating renal disease, showed values from 45 to 185 mg. per 100 cc. but "extrarenal" causes of azotemia, such as peritonitis, dehydration, hemorrhage, intestinal obstruction, or heart disease were always present. Although there seemed to be a trend toward a terminal rise of the nonprotein nitrogen, this elevation did not correlate with the severity of the renal lesion.

The urinary output varied greatly in amount but the concentration was nearly always low. Periods of oliguria were observed in 10 cases. In at least 2 of these the terminal urinary output decreased to near 200 cc. per day despite large volumes of parenteral fluid. In several of the other cases the terminal urinary output was lower than that of the immediately preceding days, but accurate figures were not always available. Accompanying this decreased urinary output there was usually a lowering of the specific gravity, as commonly happens in other types of nephrosis. Periods of polyuria, up to 4,500 cc. in 24 hours, were observed in 6 cases, but most of these appeared to be related to increased fluid intake or to the use of diuretics. The specific gravity of the urine never rose above 1.025 despite the frequent occurrence of marked dehydration. As noted above, in several instances the specific gravity was low, even during periods of oliguria. There were no significant findings in the urinary sediments.

In addition to routine parenteral fluids, hypertonic solutions in the form of 2 per cent saline were administered to 2 patients, 50 per cent glucose to a third, and 2 molar lactate to a fourth. Blood transfusions were given in 11 cases, and in one of these there was a mild reaction.

Therapeutic agents with possible nephrotoxic effects were administered in 9 cases. Seven of these patients received some type of sulfa drug and 2 received small amounts of mercupurin. Glycol poisoning from industrial exposure, ingestion of permanent antifreeze, or medicinal solvents was ruled out with reasonable certainty in all instances.

Pathologic Findings

Table III summarizes the principal post-mortem findings in the 15 cases. In 12 there was an ulcero-inflammatory process involving either the small bowel or the large bowel and occasionally both: 8 of these had nonspecific acute or chronic ulcerative colitis; the remaining 4 comprised a heterogeneous group including one case each of regional enteritis, diverticulitis, acute ulcerative ileocolitis proximal to an obstruct-

ing carcinoma of the colon, and acute ulcerative sigmoido-proctitis proximal to an obstructive lesion of the rectum caused by an infiltration as part of Hodgkin's disease. Three of the cases had enteric lesions which were not ulcerative: these were instances of subacute duodenal

TABLE III

Post-Mortem Findings

Case no.	General					Combined weight of kidneys	Tubular vacuolation
	Intestinal lesions	Extra-intestinal sepsis	Hepatic lesions	Peripheral edema	Other pertinent pathologic findings		
1	Chronic ulcerative colitis; perforation of rectum	Fecal peritonitis; acute esophagitis	Fatty infiltration, slight	o	None	310 gm.	++++
2	Chronic ulcerative colitis	Peritonitis, fibrinous	Fatty infiltration, slight	++	None	290 gm.	+++
3	Chronic ulcerative colitis; perforation of ileum	Peritonitis, purulent; bronchopneumonia	Fatty infiltration, slight	o	None	300 gm.	++
4	Acute ulcerative colitis	Bronchopneumonia	Fatty infiltration, slight	o	None	265 gm.	+
5	Chronic ulcerative colitis; rectovaginal fistula	Peritonitis, fibrinopurulent	Fatty infiltration, severe	++++	None	340 gm.	+
6	Chronic ulcerative colitis; perforation of ileum; rectovaginal fistula	Peritonitis, fibrinous; pericarditis	Fatty infiltration, moderate	+++	None	300 gm.	+
7	Chronic ulcerative colitis	None	Central hemorrhagic necrosis; fatty infiltration, slight; hemosiderosis	+	Chronic hemolytic anemia; pulmonary infarct	500 gm.	++
8	Chronic ulcerative colitis	Miliary tuberculosis	Fatty infiltration, slight; tubercles	++	None	380 gm.	++
9	Regional enteritis	None	Chronic passive congestion	++	None	350 gm.	+
10	Subacute duodenal obstruction	Peritonitis, fibrinous; cystitis	Fatty infiltration, moderate	+	None	410 gm.	+
11	Diverticulitis	Bronchopneumonia	Fatty infiltration, slight	+	Arteriosclerotic heart disease	360 gm.	+
12	Carcinoma of ascending colon; acute ileocolitis	Peritonitis, fecal	Fatty infiltration, marked	+++	None	330 gm.	+
13	Ulcerative sigmoido-proctitis; perforation of ileum; ileocolic fistula; Hodgkin's infiltration	Peritonitis, fibrinous	Fatty infiltration, slight	o	Generalized Hodgkin's disease; osteosclerosis; aplastic anemia	250 gm.	++
14	Acute enteritis	Abscess of thigh; otitis media; bronchopneumonia	Fatty infiltration, marked	+	Mesenteric lymphadenitis	150 gm.	++
15	Carcinomatosis; infiltration of bowel serosa	Cholangitis, subacute; pleuritis, fibrinous	Tumor infiltration; subacute cholangitis	+	Carcinomatosis	75 gm. (right only)	++

obstruction, minimal carcinomatous infiltration of the intestinal serosa, and nonspecific, infantile enteritis.

Some form of bacterial infection probably was present in every case although confirmatory bacteriologic studies were often lacking. Thus

Renal								
Necrosis of tubular epithelium	Regeneration of tubular epithelium	Tubular dilatation	Lower nephron atrophy	Tubular casts	Interstitial edema	Interstitial infiltration	Interstitial fibrosis	Associated renal disease
++	+	o	+++	+	+	+	+++	o
++	++	++	+	+	+	o	o	o
+	+	++	o	+++	o	++	++	o
+	±	++	+	++	+	o	o	o
+	+	+	+	+	+	o	o	o
+	+	+	+	+	o	o	o	o
+	±	+	+	++	+	+	+++	Hemosiderosis
++	+	++	o	+	o	o	o	Tuberculosis
+	+++	+++	+	+	+++	+	o	Congenital double ureter, right
++	++	++	++	++	++	+	+	Congenital double ureter and hydro-ureter, right
+	+	++	+	+	+	+	+	Nephrosclerosis
+	±	+	++	+	o	+	o	Hydropic "sucrose-type" nephrosis
+	±	++	++	++	+	++	+++	Hydronephrosis with atrophy; Hodgkin's infiltration, left kidney
+	++	++++	o	+	++	o	o	o
+	++	+++	++	+++	±	++	++	Nephrectomy, left; nephrosclerosis

among the 12 cases of ulcero-inflammatory intestinal disease which were at least secondarily invaded by bacteria, there were 7 instances of peritonitis and 2 of bronchopneumonia, as well as various minor infections such as cystitis and esophagitis. The 3 cases without intestinal ulcer were complicated by peritonitis, bronchopneumonia, and subacute cholangitis, respectively, and also had various minor focal infections.

The third of the constantly present pathologic features was some type of hepatic lesion. In all but 2 instances, however, this consisted only of fatty change which was frequently minimal. In the remaining 2 cases there was chronic passive congestion and carcinomatous infiltration, respectively. There was no correlation between the severity of the hepatic lesions and that of the renal vacuolation.

Peripheral edema, increased peritoneal and pleural fluid, and edema of the viscera, although reported as present in all of their cases by Jaffé and Sternberg,[1] were not constantly found in our series. Peripheral edema, the most common finding, was absent in 4 cases and where present varied greatly in severity, bearing no relation to the degree of vacuolation. The presence of increased fluid in the serous cavities was even less common and frequently appeared to be the result of primary exudation rather than transudation. Edema of the viscera was not observed except in some of the kidneys and this will be discussed later in this report.

Various renal abnormalities were present in 8 patients. Two of these had unilateral double ureters and one had hydronephrosis secondary to ureteral obstruction. Nevertheless, the vacuolar nephrosis was always equally severe in both kidneys. Among the remaining 5 cases there were 2 instances of moderate nephrosclerosis, and one each of tuberculosis, hydropic "sucrose-type" nephrosis, and hemosiderosis secondary to an acute hemolytic anemia.

Aside from the associated renal abnormalities, gross changes in the kidneys were slight or absent. The picture of interstitial edema with increase in weight, and pale, widened cortices as described by Jaffé and Sternberg,[1] was present in less than one-half of our cases, and in several instances the kidneys were small and congested. The combined weight of the kidneys ranged from 250 to 500 gm., with an average of 337 gm.,* which is slightly greater than normal but several of the high values were in cases complicated by other types of renal disease.

The striking changes in the kidneys were microscopic. In every in-

* Excluding case 14 which was an infant and case 15 in which only one kidney was present.

stance there was a distinctive vacuolation of the epithelial cells lining the proximal convoluted tubules (Figs. 1 and 2). This process was equally distributed throughout both kidneys but varied widely in severity from one case to another. In case 1 almost every proximal tubule which was not atrophic was involved, but more commonly some clusters of tubules, presumably belonging to the same nephron, were affected more than others, thus producing a characteristic patchy histologic picture (Figs. 7, 8, and 9). The localization of the vacuoles within individual nephrons must remain tentative since no attempt was made to study isolated nephrons either by the maceration technic of Oliver[6] or by serial sectioning. Nevertheless, it appeared fairly conclusive that the vacuolation started in the convoluted portion of the proximal tubules and, in severe instances, involved the entire length of these tubules from the neck-pieces down to the terminations of the straight segments (Fig. 5). Even the parietal lining of Bowman's capsule was vacuolated in some instances in which the tubular epithelium extended into the capsule. In the distal convoluted tubules vacuoles were rare and tended to be much smaller than those in the proximal convoluted tubules.

Characteristically, the vacuoles were well defined, rounded, clear, solitary, and so large that they distended the cells which contained them, displacing most of the cytoplasm, pushing the nucleus basally, and causing the free cell margin to bulge out into the tubular lumen. In case 1 the involvement was so severe that the tubular lumina were frequently almost completely obliterated by these "ballooned-out" epithelial cells (Fig. 2). In the less severe cases it was common to find normal appearing cells next to those which were enormously distended by vacuoles (Fig. 6). The smallest solitary vacuoles were always juxtanuclear, suggesting an origin in the Golgi zone. Some of the medium-sized vacuoles, however, had outlines coresponding to the shapes of the cells which contained them, giving the impression that the vacuole had been formed by a simple dissolution of the central portion of the cytoplasm. Finally, there were cells which contained numerous vacuoles of varied sizes which appeared to be coalescing in places. In no case, however, did there appear to be a transition from a diffuse hydropic swelling to the characteristic large, clear vacuoles. The "sucrose-type" of nephrosis present in case 12 was an apparently unrelated process.

The contents of the vacuoles failed to stain with hematoxylin and eosin, phloxine and methylene blue, Weigert-van Gieson, Sudan IV, or the periodic acid leukofuchsin method for glycogen,[7] after fixation with both Zenker's solution and formalin. These data indicate that the

vacuoles contained no appreciable amounts of protein, fat, or glycogen.* Thus it is probable that the vacuoles contained either a watery solution or some water-soluble solid other than glycogen. A pale, yellowish, fluorescent pigment which was present in some of the vacuoles of case 10 (Fig. 6) might represent the remnants of such a substance. This pigment did not stain with Sudan dyes and did not give a positive Prussian blue test.

The relatively good preservation of the nuclei even when displaced toward the base or sides of the cells by large vacuoles (Fig. 5) was another distinctive feature of this condition. While pyknosis and karyolysis with accompanying coarsely granular, acidophilic degeneration of the cytoplasm were present to some extent in every instance, such dead or dying cells rarely contained vacuoles and in general tended to occur in tubules which showed little or no vacuolation. An exception to this general rule was case 1, in which pyknosis and karyolysis were common in the most heavily vacuolated tubules (Fig. 2). This may indicate that, in its most severe form, the vacuolation can result in cell death. Other changes common in degenerating tubules, such as colloid droplet degeneration and fatty infiltration, were conspicuous by their absence.

Tubular regeneration, in the form of elongated, flattened, hyperchromatic lining cells which were occasionally multinucleated, was noted in the proximal convoluted tubules of 11 cases and was equivocal in the remaining 4. However, it was generally inconspicuous and did not correlate with the extent of tubular necrosis or vacuolation. In the distal convoluted tubules regeneration was frequently pronounced (Figs. 1 and 2) although vacuolation and necrosis were minimal or absent.

Dilatation of the lumina of the proximal convoluted tubules was present in all but one instance but again failed to correlate with the severity of vacuolation. In case 14, the 7-months-old infant, the external diameter of the tubules as well as of the lumen appeared to be enlarged. The tubular lumina were typically filled with a granular, foamy débris in which at times spheroidal or cone-shaped vesicle-like structures could be discerned (Fig. 4). Such structures might be interpreted as the desquamated delicate luminal portions of tubular epithelial cells.[8]

The collecting tubules and the lower portions of the nephrons including the thin and thick segments of Henle's loops and the distal convoluted tubules were usually somewhat atrophic, with low, granular, basophilic epithelium and occasional hyaline or granular casts. This change was particularly striking in case 1 in which the tubular lumina

* Although neither formalin nor Zenker fixation is an ideal method for the preservation of glycogen, when large amounts are present, some is generally still demonstrable by the very sensitive periodic acid leukofuchsin technic.[7]

in the medullary portions were markedly narrowed. Again, however, there was no consistent correlation with the severity of vacuolation. Several patients who had received numerous blood transfusions showed occasional pigment casts and other "irritating" casts which in some instances had produced a slight necrotic or inflammatory reaction.

Interstitial edema was thought to be present in 10 cases. It did not seem to be correlated with either the severity of peripheral edema or with that of the vacuolation. In most instances it had no obvious anatomical basis such as urinary obstruction or acute inflammation. However, the estimation of interstitial edema is notoriously unreliable, especially in the presence of interstitial fibrosis. The fibrosis was usually accompanied by slight lymphocytic infiltration and, except for several instances of nephrosclerosis with radial scarring, was most marked in the pyramids and medullary rays and sometimes in the subcapsular zone of the cortex. The most severe interstitial fibrosis was present in cases 7 and 13 which were complicated by hemosiderosis of the kidneys and hydronephrosis, respectively. In case 1, however, which apparently represented an uncomplicated vacuolar nephrosis, it was also striking (Fig. 3).

Discussion

Distinguishing Features of the Renal Vacuolation; Comparison with Similar Lesions Reported in the Literature

The renal vacuolation exemplified by the 15 cases herein reviewed represents a fairly well circumscribed pathologic entity. It seems to occur only in association with a chronic illness that is accompanied by electrolyte imbalance, malnutrition, anemia, and probably sepsis. Gastro-intestinal disorders are usually the primary process but may be subclinical. The distinctive features are the microscopic findings in the kidneys and consist of: (1) the presence in the proximal convoluted tubular epithelium of well defined, clear vacuoles which are frequently so large that they "balloon-out" the cells which contain them; (2) the failure of these vacuoles to stain by any of the usual methods, including technics for fat and glycogen; (3) the basal displacement of the nuclei with relatively good preservation even in severely involved cells; (4) the patchy distribution of the vacuolation, affecting some tubules much more than others and frequently involving certain cells to the exclusion of adjacent ones; (5) the absence or very infrequent occurrence of the vacuoles anywhere but in the proximal convoluted tubules.

Relatively few cases with similar renal lesions are mentioned in the literature. Among these only the ones described by Jaffé and Sternberg[1] and Ch'in and Hu[2] can be accepted readily as examples of the same

type of vacuolation. A case associated with "gastroenterocolitis" discussed by Williams and MacMahon[9] might possibly belong in the same category. Tokoro's case[10] of vacuolar nephrosis and stasis enterocolitis is suggestively similar but the presence of severe vacuolation in the distal as well as the proximal tubules is not consistent with our observations. The vacuolar degeneration of the proximal convoluted tubules reported by Cooke, Barclay, Govan, and Nagley[11] in a case of osteoporosis, low serum phosphorus, and renal glycosuria could not be classified conclusively because of considerable post-mortem autolysis. The 3 cases of idiopathic hydropic tubular degeneration mentioned by Bell[12] may be eliminated since they showed none of the characteristic "ballooning" of the affected cells. The nephrosis of diethylene glycol or dioxane poisoning[3] appears to be very similar to the most severe renal lesions which we have described, but this condition is generally accompanied by cortical hemorrhage and necrosis as well as by hydropic vacuoles in other organs such as the liver.

Speculations Concerning the Nature of the Vacuoles

The nature of the vacuoles and their contents is obscure. That they seemed to arise in the region adjacent to the nucleus on its luminal side and that (case 10) in some of the vacuoles a pigment appeared to have been segregated, might indicate that they are enormously enlarged Golgi vacuoles or are similar to the neutral red vacuoles which were observed in degenerating tissue cultures by Lewis and Lewis.[13] The vacuoles produced by Trowell[14] in liver cells subjected to anoxia or increased intrasinusoidal pressure may also represent a related phenomenon, but vacuoles of these types have never been described as reaching such a size that they actually distended the cells which contained them.

The vacuolar contents apparently is either a watery solution or some water-soluble solid other than glycogen. The above-mentioned pigment might have been the remains of such a substance which had withstood the solvent action of the aqueous fixative. On the other hand, Jaffé and Sternberg[1] observed the vacuoles in unfixed, teased preparations as "clear, refractile droplets." This would seem to justify their assumption that the vacuolation was "hydropic."

Evaluation of Clinical and Experimental Findings as Possible Pathogenic Factors

Although clinical study of our cases revealed no single feature which might be considered a specific etiologic factor, the cases were sufficiently similar to suggest a common mechanism of pathogenesis. Such a mecha-

nism might be the result of several interacting factors, none of which by itself would have been sufficient to produce the lesions. This possibility is made more plausible by the fact that many conditions present in severe intestinal disease are known to result in renal tubular injury. Such of these conditions as might have existed in the present cases will be discussed briefly in the light of the available experimental data.

The literature regarding nephropathic factors which might be related to the formation of the hydropic vacuoles is summarized in Table IV. These many factors are diverse but most of them fall into the categories of nutritional deficiencies, fluid-electrolyte imbalance, and "toxic" conditions; they will be discussed in that order.

That *nutritional disturbances* may result in renal vacuolation is indicated by the work of Suzuki[15] who found "cellular disintegration with occasional vacuole formation" in the renal tubules of a variety of experimental animals after combined food and water deprivation. Moreover, Sjöstrand[16] observed vacuole-like tubular lesions in mice after either food or water deprivation alone. These observations may be of interest because in most, if not all, of our cases some degree of starvation and/or dehydration was present. Bell and Knutson,[27] however, found no tubular lesions in 6 patients whose azotemia they attributed to decreased fluid intake. Nevertheless one of 3 cases with diarrhea causing dehydration and azotemia showed a "severe hydropic degeneration" of the renal tubules. Specific nutritional deficiencies such as hypoproteinemia[17] and choline deficiency[18,19] have been reported to produce tubular damage and may have played a rôle in the development of the vacuolar nephrosis.

Disorders in electrolyte balance were prominent in most of our cases, and similar disorders such as ammonium chloride acidosis[20] and dietary deficiencies of potassium[21] or chlorides[22] have been reported to damage the proximal convoluted tubules of the kidney. With ammonium chloride administration the development of hydropic vacuoles was observed,[20] but in other types of acidosis the kidneys appeared to remain unchanged.[28] Also, in at least 2 of our cases alkalosis rather than acidosis existed terminally. Moreover, in case 14, there was no evidence that any pronounced imbalance of electrolytes had been present.

The parenteral administration of 2 per cent saline, 8 to 24 per cent glucose, or 10 per cent urea solutions has been found to produce vacuole-like lesions in the proximal tubules of mice.[16] Moreover, hypertonic sucrose and certain other sugar solutions are known to cause a diffuse, granular swelling of the proximal tubular epithelium in human patients, indicating a possible relationship between diffuse and vacuolar hydropic

TABLE IV

Nephropathic Factors Possibly Related to the Vacuolar Nephrosis

Nephropathic conditions	Lesions in proximal convoluted tubules	Animal species	References
Deprivation of water and food (17 days)	Granular disintegration and occasional vacuole formation	Rabbits, guinea-pigs, rats, mice	Suzuki[15]
Deprivation of food (12 to 54 hours)	Vacuole-like spaces with fluorescent linings	Mice	Sjöstrand[16]
Deprivation of water (36 to 72 hours)	Vacuole-like spaces with fluorescent linings	Mice	Sjöstrand[16]
Hypoproteinemia (plasmapheresis)	Cloudy swelling, fatty infiltration, occasional necrosis and scarring	Dogs	Barker and Kirk[17]
Choline deficiency	Granular, hyaline, and droplet degeneration; from fatty change to symmetrical hemorrhagic cortical necrosis	Rats	Christensen[18] Hartroff[19]
Acidosis (NH_4Cl, 0.5 to 1.0 gm. per kg. per day)	Vacuolation; dilatation of lumina	Rabbits	Govan and Parkes[20]
Potassium deficiency (dietary)	Fatty change, hyaline necrosis, dilatation	Rats	Follis, Orent-Keiles, and McCollum[21]
Chloride deficiency (dietary)	Epithelial swelling, degeneration, and eventual scar tissue replacement	Rats (3 to 4 weeks old)	Cuthbertson and Greenberg[22]
Injection of hypertonic solutions of saline, glucose, or urea (intravenous)	Vacuole-like spaces with fluorescent linings	Mice	Sjöstrand[16]
Injection of hypertonic sugar solutions (parenteral)	Hydropic swelling	Human	Anderson[23] Rigdon and Cardwell[24]
Diethylene glycol toxicosis	Hydropic vacuolation with "ballooning" of cells, pyknosis, and karyolysis; frequently complicated by symmetrical cortical necrosis	Human, rats, rabbits, dogs	Geiling and Cannon[3]
Unilateral ureteral ligation	Atrophy, dilatation; occasional vacuolar nephrosis	Rabbits	Mallory, Crane, and Robbins[25]
Clamping of renal artery followed by restoration of circulation:		Rat	Emmel[26]
(a) After 10+ minutes of ischemia	Enspherulation and clumping of mitochondria; hydropic vacuolation		
(b) After 20+ minutes of ischemia	"Distention" of tubules and interstitial edema in addition to changes in (a)		
(c) After 45+ minutes of ischemia	Necrosis and desquamation of epithelium in addition to changes in (a) and (b)		

change. Patchy areas of the "sucrose-type" lesions were present in the absence of hypertonic therapy in one of our cases as well as in a case of enterocolitis with diarrhea mentioned by Anderson.[23] It should be noted, however, that no transitions from the vacuolar lesions into the "sucrose-type" were ever observed in our cases.

The intake of diethylene glycol or certain of its ether derivatives has also been known to cause large, hydropic vacuoles in the renal tubules.[3] While no such drug could be implicated in our cases, it is possible that some toxin absorbed from the bowel or from a septic focus might have acted in a similar way. Certainly the similarity of these lesions to the ones we have described warrants further comparative study.

The experimental lesions perhaps most strikingly similar to our cases were those discovered by Mallory, Crane, and Robbins[25] in the hydronephrotic kidneys of rabbits which had had one ureter ligated 6 to 21 days previously. Such lesions had not been described by Suzuki[15] who had performed similar experiments, but in several ureteral ligations performed in rabbits by us, small foci of such vacuolation were found. The form and distribution of the vacuoles were almost identical to those in the human kidneys (Fig. 10); they were of comparable size, frequently "ballooning out" the cells which contained them; they occurred chiefly if not entirely within the proximal convoluted tubules; the smaller, solitary vacuoles conformed to the outlines of the cells in which they were present; the severity of vacuolation was decreased in the atrophic subcapsular tubules; the generally good preservation of the nuclei was striking. Even the occurrence of yellow, fluorescent pigment within some of the vacuoles, as described in case 10, was duplicated in several of the hydronephrotic rabbits. On the other hand, the nuclei of the vacuolated cells were frequently pushed toward the apex of the cell rather than toward the base; the proximal tubules were never dilated; necrosis and regeneration of the epithelium were minimal or absent.

A possible relation between the lesions produced after ureteral obstruction and many of the other vacuolar lesions mentioned above is indicated by the recent work of Sjöstrand[16] who investigated the kidney by means of fluorescent microscopy during normal diuresis as well as during a variety of conditions which placed an increased demand on the reabsorptive capacity of the tubular epithelium. He found small vacuoles with yellowish fluorescent linings in about 5 per cent of normal mice, but after intravenous injections of various hypertonic solutions, as well as after prolonged starvation or deprivation of fluid, these vacuoles became more common, larger, and associated with fluorescent cytoplasmic pigment. These changes are quite similar to those seen in the ureteral

ligation experiments[25] and it would be important to determine if these characteristic pigmentary changes are found also in some of the other vacuolar lesions discussed.

Finally, we must consider temporary cortical ischemia as a possible cause of the tubular vacuolation. Emmel[26] has shown that "watery" vacuoles appear in the proximal convoluted tubules of rats during the period of recovery after clamping the renal artery for 10 or more minutes. Judging from the photomicrograph in his paper, these vacuoles did not resemble the ones found in our cases either in shape or cytoplasmic localization. It is of interest, however, that by prolonging the period of renal ischemia up to 1 hour and allowing for a period of "recovery" it is possible to reproduce some histologic findings commonly associated with the giant hydropic vacuoles, including tubular dilatation, interstitial edema and fibrosis, necrosis and desquamation of tubular epithelial cells, and cast formation. A possible mechanism for the production of cortical ischemia in our cases is suggested by the work of Trueta, Barclay, Franklin, Daniel, and Pritchard[29] who showed that bacterial toxins, circulatory shock, and anti-diuretic hormone may all produce cortical vasospasm.

From the data reviewed it is seen that a variety of nephropathic disorders present in any one case might possibly have been concerned in producing tubular vacuolation. It should be emphasized, however, that most of the experimental lesions attributed to these disorders did not reach the size of the human lesions we have described. Moreover, the nonspecificity of these disorders, their inconstancy from one case to another, and their failure to correlate quantitatively with the severity of the renal lesions all speak against the implication of any one of them alone as a specific pathogenic agent. There would seem to remain two possibilities: either there is an as yet undisclosed etiologic agent or the synergistic interaction of several nonspecific factors produced the giant vacuoles.

Summary

Fifteen cases of a distinctive nephropathy associated with chronic intestinal disease have been studied. This condition was found in 8 of 72 cases of nonspecific ulcerative colitis as well as in 7 of 80 cases of intestinal disease of various other types. The lesions were characterized by the presence in the proximal convoluted tubular epithelium of well defined, clear vacuoles which were frequently so large that they "ballooned out" the cells which contained them. These vacuoles failed to stain by any of the usual methods, including those for fat and glycogen.

The nuclei were generally displaced toward the base of the cells but remained relatively well preserved. The distribution of the vacuoles was patchy, affecting some tubules more than others and frequently involving certain cells and sparing adjacent ones in the same tubule.

The clinical findings in these cases did not conform to a consistent pattern. Even intestinal symptoms were not always significant. Nevertheless, the cases were similar in that all of the patients had been ill for more than 4 weeks and, at least terminally, they probably all developed some degree of malnutrition, anemia, hypoproteinemia, electrolyte imbalance, and sepsis. Signs and symptoms of kidney dysfunction were varied, inconstant, and failed to correlate with the severity of the vacuolation. Certain presenting nephropathic factors may have pathogenic significance. Although the etiology and pathogenesis of these renal lesions remain unclear, the coexistence of intestinal disturbances with these tubular changes suggests a possible causal relationship.

We are indebted to Mr. Leo Goodman, photographer at Boston City Hospital, for the photomicrographs appearing in this article.

ADDENDUM

Since the completion of this manuscript, the coexistence of vacuolar nephropathy and ulcerative colitis has been reported by Jensen, E. J., Baggenstoss, A. H., and Bargen, J. A., *Am. J. M. Sc.*, 1950, **219**, 281–290. Their findings agree with our own. An additional case report: Intestinal lipodystrophy (Whipple's disease) occurring with parathyroid hyperplasia and nephrosis. Report of a case with autopsy, Odessky, L., and Burdison, W. R., *Arch. Path.*, 1950, **49**, 307–320, also describes a similar renal lesion.

REFERENCES

1. Jaffé, R. H., and Sternberg, H. Über die vakuoläre Nierendegeneration bei chronischer Ruhr. *Virchows Arch. f. path. Anat.*, 1919–20, **227**, 313–319.
2. Ch'in, K. Y., and Hu, C. H. Pathological lesions caused by *B. dysenteriae*. A study of 89 autopsied cases. *Chinese M. J.*, 1940, Suppl. III, 120–144.
3. Geiling, E. M. K., and Cannon, P. R. Pathologic effects of elixir of sulfanilamide (diethylene glycol) poisoning. *J. A. M. A.*, 1938, **111**, 919–926.
4. Cabot case 27032. *New England J. Med.*, 1941, **224**, 120–122.
5. Cabot case 19101. *New England J. Med.*, 1933, **208**, 546–552.
6. Oliver, J. New directions in renal morphology; method, its results and its future. *Harvey Lect.*, 1945, **40**, 102–155.
7. Lillie, R. D. Studies on the preservation and histologic demonstration of glycogen. *J. Tech. Methods*, 1947, **27**, 23–61.
8. Krüger, O. Versuche zur Fixierung des Funktionszustandes der Hauptstücke der menschlichen Niere und zur Bedeutung der Kuppenbläschen der Epithelien. *Ztschr. f. mikr.-anat. Forsch.*, 1937, **41**, 453–468.
9. Williams, R. H., and MacMahon, H. E. Clinicopathological conference: gastroenterocolitis; carcinoma of pancreas; "clear-cell" nephrosis. *Bull. New England Med. Center*, 1947, **9**, 274–283.

10. Tokoro, Y. Zur Frage der vakuoligen Zelldegeneration der Niere. *Tr. Soc. path. jap.*, 1938, **28**, 297–304.

11. Cooke, W. T., Barclay, J. A., Govan, A. D. T., and Nagley, L. Osteoporosis associated with low serum phosphorus and renal glycosuria. *Arch. Int. Med.*, 1947, **80**, 147–164.

12. Bell, E. T. Renal Diseases. Lea & Febiger, Philadelphia, 1946, pp. 261–262.

13. Lewis, W. H., and Lewis, M. R. Behavior of Cells in Tissue Cultures. In: Cowdry, E. V. (ed.) General Cytology. University of Chicago Press, Chicago, 1924, p. 427.

14. Trowell, O. A. The experimental production of watery vacuolation of the liver. *J. Physiol.*, 1946, **105**, 268–297.

15. Suzuki, T. Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen. Gustav Fischer, Jena, 1912, 244 pp.

16. Sjöstrand, F. Über die Eigenfluoreszenz tierischer Gewebe mit besonderer Berücksichtigung der Säugetierniere. *Acta Anat.*, 1945–46, Suppl. 1, **1**, 137–138.

17. Barker, M. H., and Kirk, E. J. Experimental edema (nephrosis) in dogs in relation to edema of renal origin in patients. *Arch. Int. Med.*, 1930, **45**, 319–346.

18. Christensen, K. Renal changes in the albino rat on low choline and choline-deficient diets. *Arch. Path.*, 1942, **34**, 633–646.

19. Hartroff, W. S. Histological changes in kidneys of choline-deficient rats. (Abstract.) *Anat. Rec.*, 1947, **97**, 342.

20. Govan, A. D. T., and Parkes, J. Observations on experimental ammonium chloride acidosis. *J. Path. & Bact.*, 1946, **58**, 505–515.

21. Follis, R. H., Jr., Orent-Keiles, E., and McCollum, E. V. The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium. *Am. J. Path.*, 1942, **18**, 29–39.

22. Cuthbertson, E. M., and Greenberg, D. M. Chemical and pathological changes in dietary chloride deficiency in the rat. *J. Biol. Chem.*, 1945, **160**, 83–94.

23. Anderson, W. A. D. Sucrose nephrosis and other types of renal tubular injuries. *South. M. J.*, 1941, **34**, 257–263.

24. Rigdon, R. H., and Cardwell, E. S. Renal lesions following the intravenous injection of a hypertonic solution of sucrose. *Arch. Int. Med.*, 1942, **69**, 670–690.

25. Mallory, G. K., Crane, A. R., and Robbins, S. L. Personal communication.

26. Emmel, V. M. Mitochondrial and pH changes in the rat's kidney following interruption and restoration of the renal circulation. *Anat. Rec.*, 1940, **78**, 361–381.

27. Bell, E. T., and Knutson, R. C. Extrarenal azotemia and tubular disease. *J. A. M. A.*, 1947, **134**, 441–446.

28. Addis, T., MacKay, E. M., and MacKay, L. L. The effect on the kidney of the long continued administration of diets containing an excess of certain food elements. II. Excess of acid and of alkali. *J. Biol. Chem.*, 1926–27, **71**, 157–166.

29. Trueta, J., Barclay, A. E., Franklin, K. J., Daniel, P. M., and Pritchard, M. M. L. Studies of the Renal Circulation. Charles C Thomas, Springfield, Ill., 1947, 187 pp.

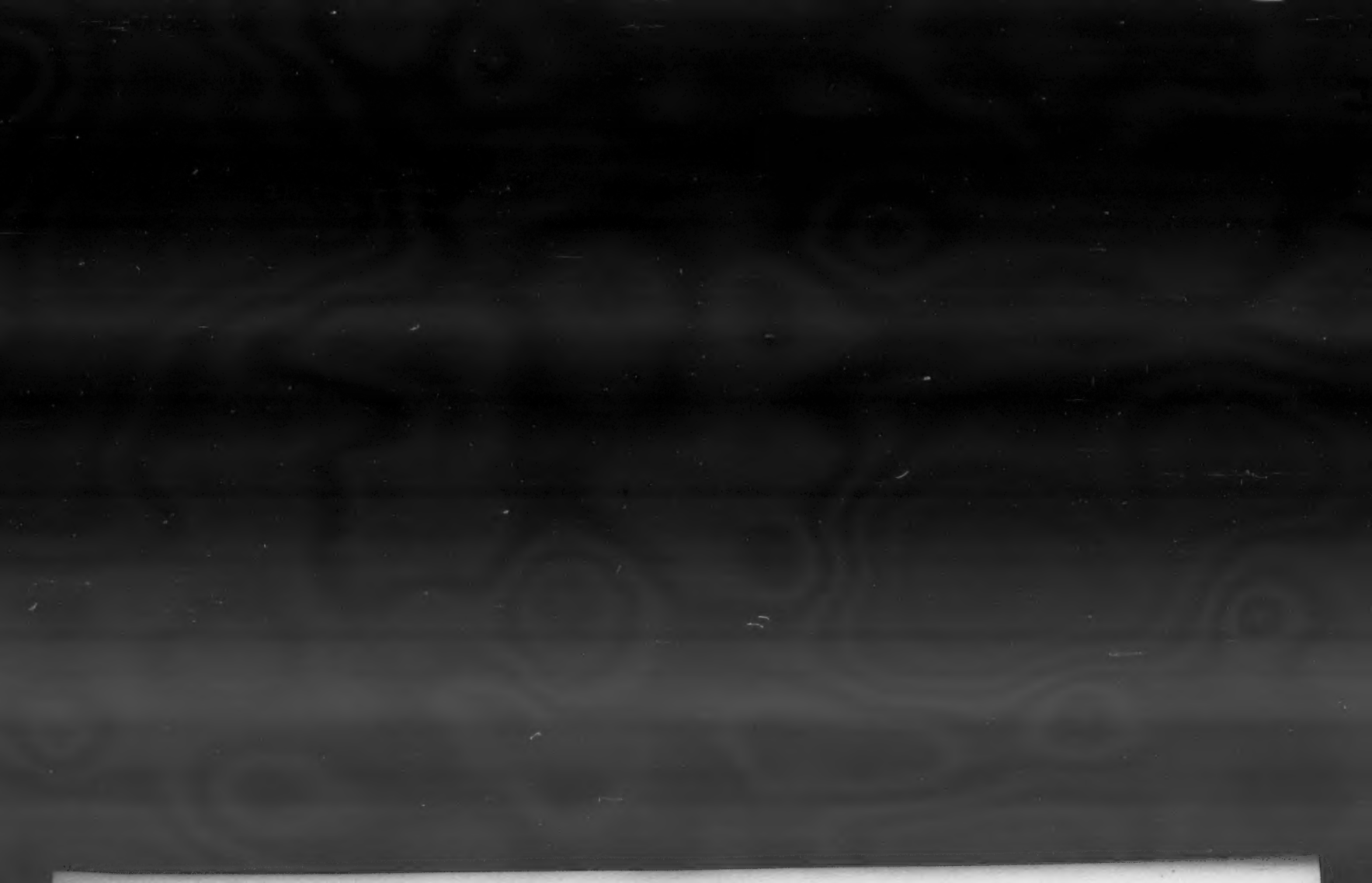
[*Illustrations follow*]

DESCRIPTIONS OF PLATES

PLATE 56

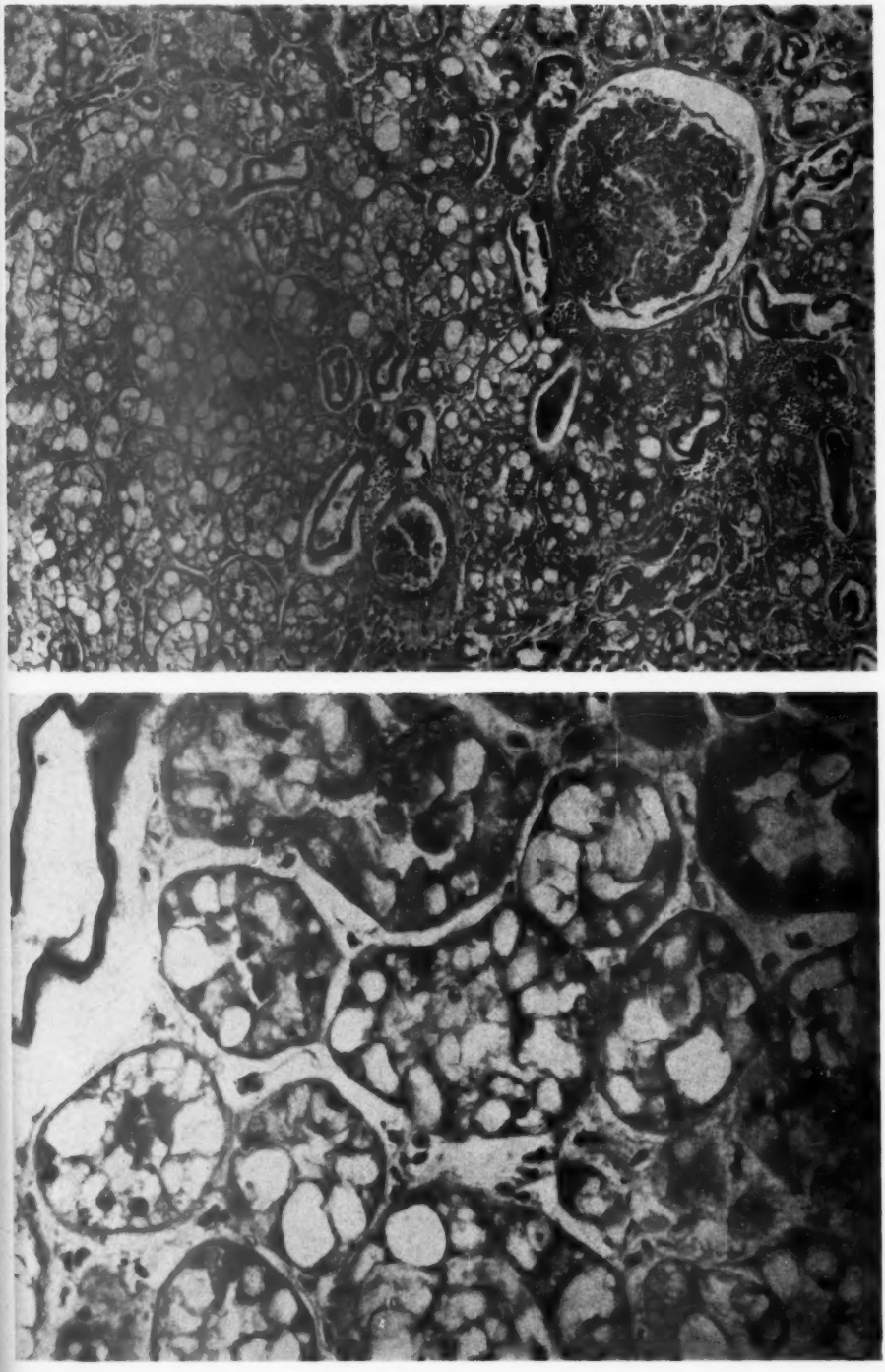
FIG. 1. Case 1 (Massachusetts General Hospital no. 10567). Extreme vacuolation of the proximal convoluted tubules. Their lumina are frequently almost obliterated by the "ballooned-out" epithelial cells. The intercalated segment of a distal convoluted tubule at the vascular pole of the large glomerulus is lined by hyperchromatic epithelium which is free of vacuoles and appears to be regenerating. Similar tubules scattered through the section probably represent other distal convoluted tubules. Phloxine and methylene blue stain. × 490.

FIG. 2. Case 1. High power view from the same section as used for Figure 1. The nuclei of the vacuolated cells are usually pushed toward the base of the cells and there is considerable pyknosis and karyolysis. Some nuclei, however, are well preserved despite the presence of large vacuoles in the same cells. The partly obliterated tubular lumina contain foamy débris and occasional necrotic desquamated cells. Phloxine and methylene blue stain. × 805.



Ам

Ku

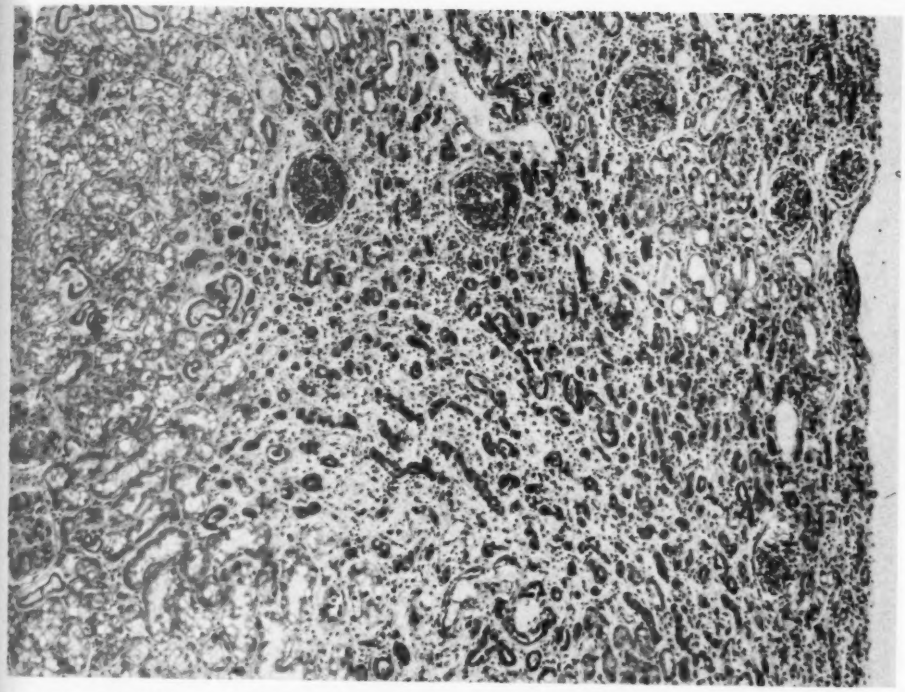


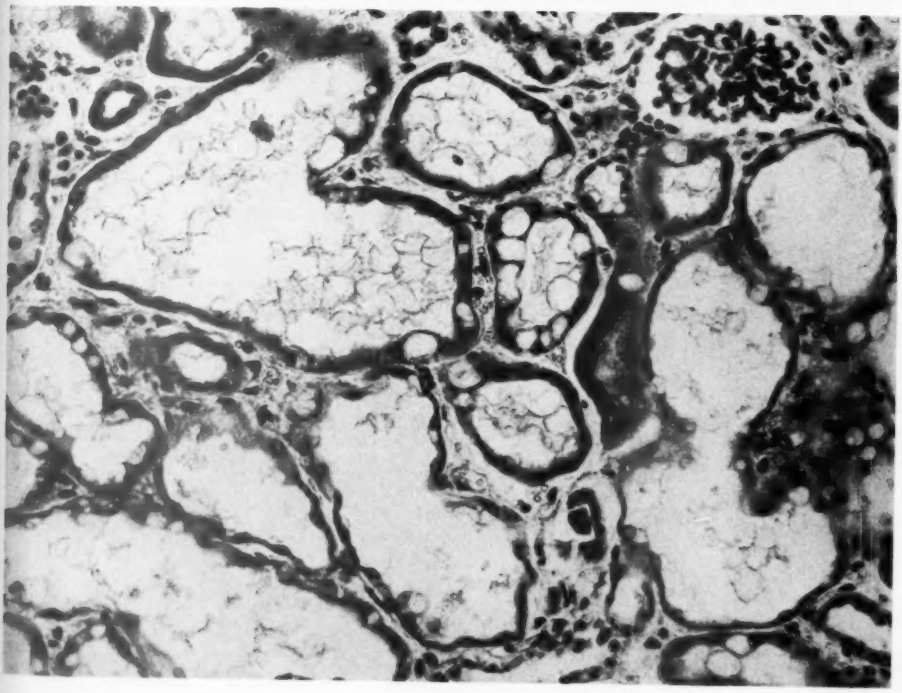
Kulka, Pearson, and Robbins Vacuolar Nephropathy

PLATE 57

FIG. 3. Case 1. Severe vacuolar nephrosis and parenchymal atrophy. There is marked vacuolation of the proximal tubules in the lower portion of the field while the tubules in the peripheral cortex and the medullary rays are atrophic and collapsed. Phloxine and methylene blue stain. × 90.

FIG. 4. Case 14 (Boston City Hospital no. A-47-475). Vacuolar nephrosis and marked tubular dilatation in a case of infantile diarrhea. The size of the proximal convoluted tubules may be compared with that of the glomerulus. In some instances the external as well as the internal diameter of the tubules is enlarged. Phloxine and methylene blue stain. × 670.



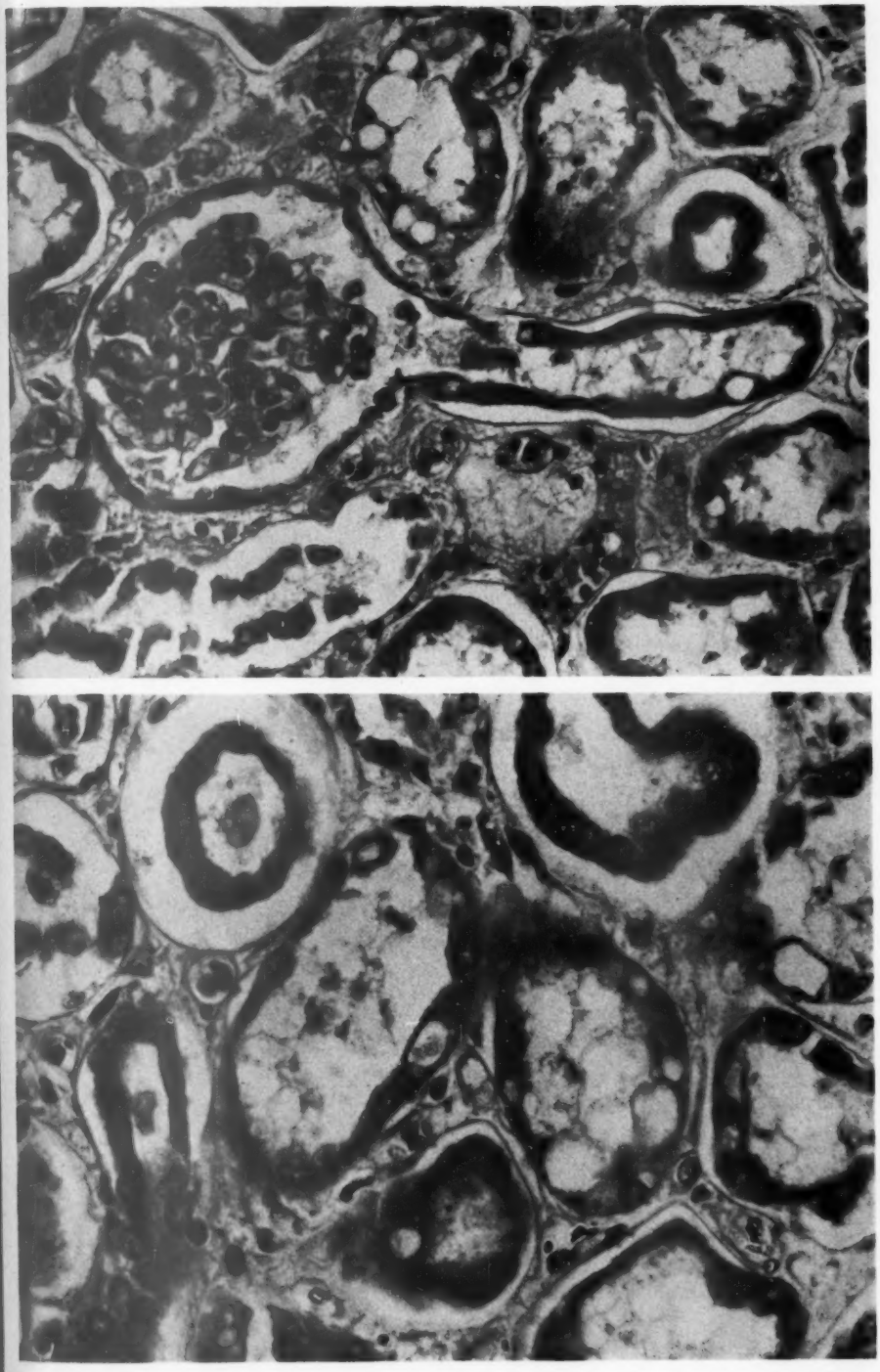


Kulka, Pearson, and Robbins Vacuolar Nephropathy

PLATE 58

FIG. 5. Case 10 (M.G.H. no. 11340). Moderate vacuolar nephrosis with associated epithelial necrosis and regeneration. The vacuoles are seen to extend into the neck of a proximal tubule which also shows absence of nuclei on one side and elongated, hyperchromatic, apparently regenerating cells on the opposite side. Phloxine and methylene blue stain. × 805.

FIG. 6. Case 10. Moderate vacuolar nephrosis with associated epithelial necrosis and regeneration. The epithelium of the central tubule is flattened and the nuclei are large and hyperchromatic. The large oblong vacuole in the same tubule contains some faintly staining, amorphous pigment. Phloxine and methylene blue stain. × 895.



Kulka, Pearson, and Robbins Vacuolar Nephropathy

PLATE 59

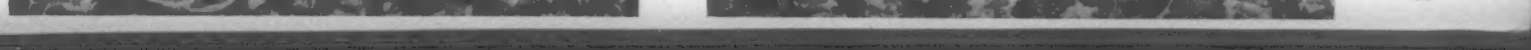
Figs. 7 to 10. Moderate vacuolar nephrosis in a variety of disease states. All are shown at the same magnification to illustrate their essential similarity.

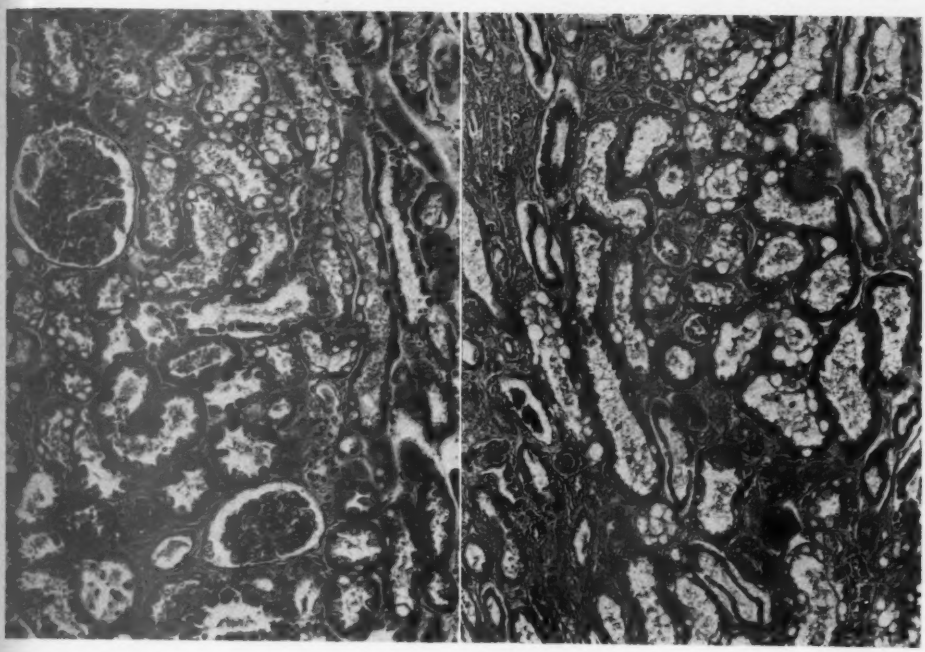
FIG. 7. Case 6 (B.C.H. no. A-40-828). Moderate vacuolar nephrosis in a case of ulcerative colitis. Phloxine and methylene blue stain. × 155.

FIG. 8. Case 13 (M.G.H. no. 8556). Moderate vacuolar nephrosis in a case of generalized Hodgkin's disease which had resulted in bilateral ureteral obstruction and hydronephrosis as well as in partial obstruction of the rectum and secondary ulcerative sigmoidoproctitis. Phloxine and methylene blue stain. × 155.

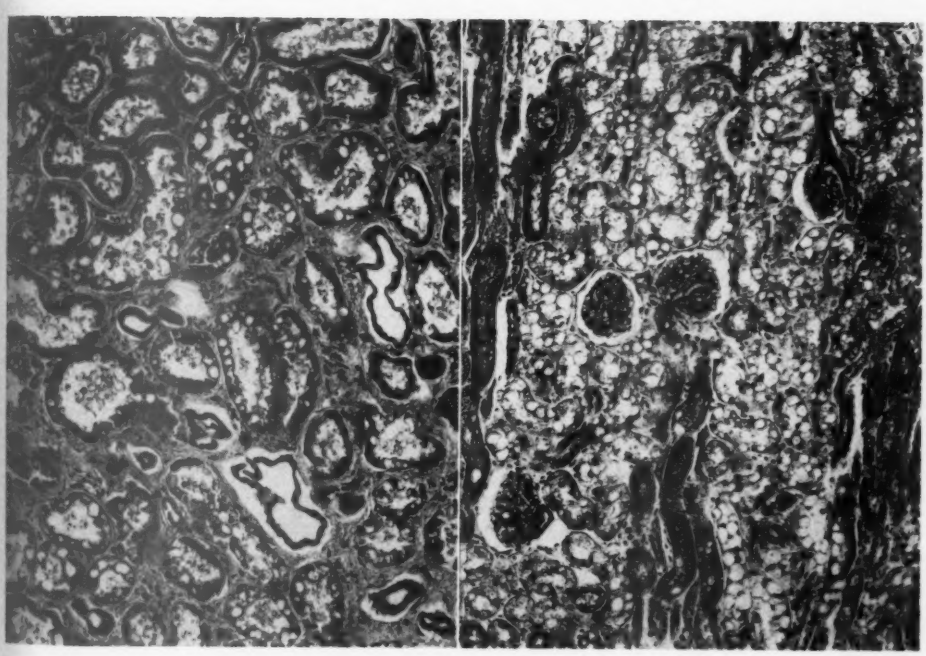
FIG. 9. Case 15 (M.G.H. 6728). Moderate vacuolar nephrosis in a case of carcinomatosis with microscopic infiltration of the bowel serosa but no clinical manifestations of intestinal disease. Phloxine and methylene blue stain. × 155.

FIG. 10. Vacuolar nephrosis in the kidney of a rabbit which had had its ureter tied 21 days previously. There is a striking resemblance to the vacuolar nephrosis in the human cases of intestinal disease. Phloxine and methylene blue stain. × 155.





7 8



9 10

Kulka, Pearson, and Robbins Vacuolar Nephropathy

PARENCHYMATOUS LESIONS OF LIVER AND KIDNEY OF MICE DUE TO PECTIN*

GOETZ W. RICHTER, M.D.

(From the Department of Pathology, Cornell University Medical College, New York 21, N.Y.)

Macromolecular carbohydrates such as acacia, methyl cellulose, and pectin, when administered artificially to man and various species of animals, may give rise to a number of interesting lesions, some of which are similar to naturally occurring disease processes.[1] The pathogenicity of the pectins has been discussed especially by Hueper[2] and by Popper *et al.*,[3] who have shown that they may be deposited in various organs, notably in the sinusoids of liver and spleen and in the lumina of renal tubules in various species and that they sometimes elicit a giant cell reaction[3]; in addition Hueper has described a diffuse "foam transformation" of the hepatic cells following the intravenous injection of pectin in dogs.[2] The present report calls attention to further and hitherto undescribed lesions which developed after the intravenous administration of pectin to mice; these consist of focal necrosis of hepatic cells which had previously become loaded with pectin and of focal degenerative changes in the renal tubules.

MATERIALS AND METHOD

The pectins used were purified apple and citrus pectins, obtained from the Universal Colloid Company of New York. Two sets of solutions were used: (1) Pectin which had been "wetted" with absolute alcohol was dissolved in distilled water to make a 3 per cent solution; (2) Pectin was boiled directly in distilled water to make a 3 per cent solution which was subsequently autoclaved for 15 minutes at a pressure of 15 lbs. The final solutions were clear in both cases, and without insoluble residue.

Injections were made into the tail veins of male Swiss albino mice weighing approximately 30 gm. In the first experiment each animal received three injections, ranging from 0.5 to 2.0 cc., during a span of 2 weeks. Two mice were given autoclaved citrus pectin; 2 others, non-autoclaved citrus pectin; and 2 received autoclaved apple pectin. Immediately following the injection some of the animals had marked hyperpnea and lay on their sides, but they recovered spontaneously within 1 hour. They did not otherwise appear ill at any time although they had lost from 5 to 7 gm. in weight 1 month after the first injection.

* Received for publication, June 8, 1949.

They were killed by ether inhalation at that time. Sections were fixed in Zenker's fluid-formaldehyde solution.

In a second experiment 6 mice were given two intravenous injections of 1 cc. of autoclaved citrus pectin and 6 others received 1 cc. of autoclaved apple pectin. The second injections were given 1 week after the first. These animals were autopsied 6 weeks after the first injection.

A number of mice were given intraperitoneal injections of pectin, but none of them developed any lesions.

Results

With the exception of splenomegaly (spleen up to approximately twice normal size) in several of the animals, no gross abnormalities were observed.

The most striking lesions were seen microscopically in the liver and kidney. Most of the mice had numerous focal lesions of the liver parenchyma. These consisted of disintegrated or disintegrating liver cells which had previously become filled with a material which took a grayish blue and sometimes a purple color when stained with hematoxylin and eosin. This was shown to be pectin, as it was selectively stained a deep red by ruthenium red[2-4] (Fig. 3). Many of these cells had a vacuolation which gave them the appearance of "foam cells." Often their outline was quite well preserved, as was their normal eosinophilic material. In other areas there were only masses of pectin, containing disintegrated nuclei of liver cells (Figs. 1 and 2). Although pectin could be demonstrated also in some liver cells away from the focal areas, it was far less conspicuous there than in the focal lesions. In addition, there were granulomata in several areas where severe damage had been brought about by the pectin. In some of these areas lymphocytes and macrophages were prominent. In others there was beginning fibrosis (Fig. 4). Liver cells in various stages of mitosis were seen in and about some of these granulomata, demonstrating the regeneration of hepatic parenchyma. Pectin was also seen within liver sinusoids and inside some of the reticulo-endothelial cells.

In the kidneys pectin was demonstrated inside glomerular capillaries as well as within some of the endothelial cells themselves. It was prominent in the lumina of proximal and distal tubules but most notably in the collecting tubules. The tubular epithelium in the affected segments was frequently pale, and pectin could be stained within many but not nearly all of the cells involved. There was also a shedding of this epithelium, especially in areas where pectin had plugged the tubular lumen. Pectin casts were a prominent feature in the collecting tubules. Again,

in several of the severely affected portions there was granuloma formation (Figs. 5 and 6). The lesions in the kidneys were less abundant than those in the liver, but they were also focal, and from their general configuration one might be led to conclude that stasis of pectin was an important factor in their genesis.

The lungs of one of the animals showed foam cell formation of the type described by Hueper.[2] There was plugging of pulmonary capillaries by pectin in 2 of the animals.

In the spleens, which were enlarged, there was marked reticulum cell hyperplasia, and pectin could be demonstrated inside some macrophages. The same animals usually had a similar reaction in the lymph nodes where foam cell formation was prominent.

No remarkable changes were seen in other organs.

It should be pointed out that there was no difference in the appearance or distribution of the lesions which could be related to the fact that some of the animals received autoclaved pectin, while others received non-autoclaved pectin. Nor was there any difference between the lesions produced by apple and citrus pectins.

Discussion

It seems plain that apple and citrus pectins can produce focal necrosis in the hepatic parenchyma and degenerative changes in the renal tubules of mice. Such lesions were not reported by Hueper,[2] who worked with rabbits and dogs, nor by Popper *et al.*,[3] who studied humans and rabbits, although Hueper observed a diffuse "foam cellular transformation" of hepatic cells following the injection of pectin, and he reported similar lesions following the injection of acacia and of methyl cellulose.[5,6] Metcalf and Hawkins[7] noted the same in dogs after the injection of acacia and also reported necrosis of the liver of a diffuse type in some of their animals.

It has been said that pectin, a mixture of large carbohydrate molecules of different constitutions,[8] is probably not metabolized by the mammalian organism, since a large proportion of the injected material is excreted unchanged by the kidneys.[9] However, pointed studies to show whether or not some of it is broken down have never been done. That this does occur has been suggested.[10]

The significance of the liver lesions produced by pectin would seem to lie in their analogy to lesions produced by some natural diseases. Focal necrosis of the liver may develop in typhoid fever, tularemia, diphtheria, scarlet fever, poliomyelitis, measles, and other diseases. In diphtheria and scarlet fever these lesions are due to toxins. In typhoid fever, toxic

effects as well as stasis of inflammatory sludges in the liver sinusoids have been invoked as causative mechanisms. In the case of the two virus diseases mentioned it is possible that the macromolecular nature of the virus proteins may play an important rôle in the inability of the invaded cells to handle the virus, as Hueper[1] has suggested. Yet why the lesions are focal remains a mystery.

Stasis does not appear to be an adequate explanation for the hepatic lesions produced by pectin. It may be that different liver cells or different areas of liver cells show different degrees of functional activity, and therefore perhaps of functional vulnerability, at various times. Only cells appearing heavily laden with pectin were necrotic, suggesting that they had been more active in ingesting pectin than the healthy cells. In any event, the sequence of ingestion of pectin molecules by liver cells, focal necrosis, and finally granuloma formation offers a suggestive analogy to that which may be seen in natural diseases. Whether or not the death of cells is related to the size of the noxious agent as well as to its chemical constitution remains unsettled.

As to the changes in the renal tubules, the anatomical evidence points to stasis of pectin as a cause. That the presence of casts of various kinds within the tubules can lead to degenerative changes in the tubular epithelium is a well known fact. How or why these changes occur is a matter of hypothesis. In the present instance we know that at least some of the epithelial cells take up the pectin. But inasmuch as pectin could be demonstrated only in a minority of abnormal cells, no definite conclusions regarding the mechanism of cell alteration in the renal tubules can be deduced.

There are many "foreign" colloids which various parenchymal cells will ingest. This may represent an attempt to get rid of the material via intracellular breakdown which can result in cell death. The old concept of "storage" of such materials may well bear some revision. In the case of pectin, studies on the excretion of galacturonic acid, a constituent of pectin molecules, would provide important information in this regard. The fact that pectin is excreted unchanged in large amounts[9] does not exclude the possibility of some degree of breakdown inside the body.

Summary

Pectin, administered intravenously to mice, produces focal necrosis of the hepatic parenchyma. This appears to be related to its being taken into the affected cells in excessive amounts.

Focal degenerative changes of various degrees were seen in the renal tubules.

Granuloma formation has been observed in areas of focal necrosis in both liver and kidney.

REFERENCES

1. Hueper, W. C. Macromolecular substances as pathogenic agents. *Arch. Path.*, 1942, 33, 267–290.
2. Hueper, W. C. Experimental studies in cardiovascular pathology. VI. Pectin atheromatosis and thesaurosis in rabbits and in dogs. *Arch. Path.*, 1942, **34**, 883–901.
3. Popper, H., Volk, B. W., Meyer, K. A., Kozoll, D. D., and Steigmann, F. Evaluation of gelatin and pectin solutions as substitutes for plasma in the treatment of shock. *Arch. Surg.*, 1945, **50**, 34–45.
4. Eisen, G. Notes on fixation, stains, the alcohol method, etc. *Ztschr. f. wissensch. Mikr.*, 1897, **14**, 195–202.
5. Hueper, W. C. Experimental studies in cardiovascular pathology. V. Effects of intravenous injections of solutions of gum arabic, egg albumin and gelatin upon the blood and organs of dogs and rabbits. *Am. J. Path.*, 1942, **18**, 895–933.
6. Hueper, W. C. Experimental studies in cardiovascular pathology. IV. Methyl cellulose atheromatosis and thesaurosis. *Arch. Path.*, 1942, 33, 1–17.
7. Metcalf, R. G., and Hawkins, W. B. Plasma protein, bile salt and cholesterol metabolism as influenced by multiple injections of gum acacia in bile fistula dogs. *Am. J. Path.*, 1939, **15**, 429–444.
8. Hirst, E. L., and Jones, J. K. N. The Chemistry of Pectic Materials. In: Pigman, W. W., and Wolfrom, M. L. (eds.) Advances in Carbohydrate Chemistry. Academic Press, Inc., New York, 1946, **2**, 235–251.
9. Kozoll, D. D., Volk, B. W., Steigmann, F., and Popper, H. Pectin excretion studies in the human being. *J. Lab. & Clin. Med.*, 1946, **31**, 30–39.
10. Baier, W. E., Bryant, E. F., Joseph, G. H., and Palmer, G. H. Uronic acids in animal bodies. *Science*, 1945, **101**, 670–671.

[*Illustrations follow*]

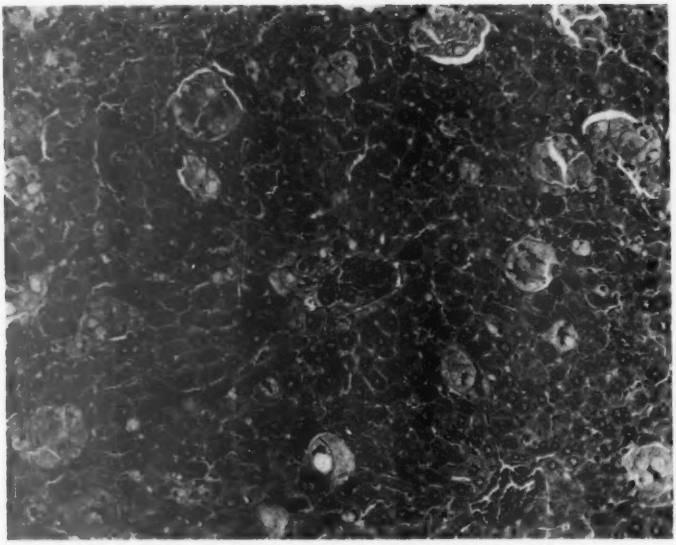
DESCRIPTION OF PLATES

PLATE 60

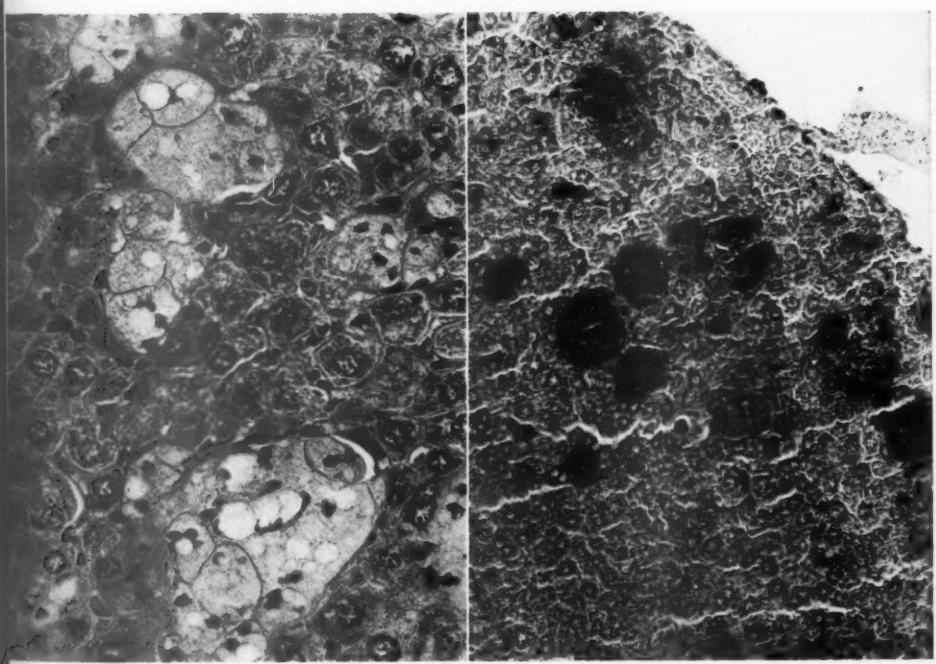
FIG. 1. Distribution of focal lesions in the liver. Hematoxylin and eosin stain. × 180.

FIG. 2. Three areas of focal necrosis in the liver from Figure 1. The cells in these areas are loaded with pectin and are disintegrating. The nuclei are quite pyknotic. The cytoplasm took a grayish blue stain but retained many eosinophilic granules. A number of damaged liver cells which took the stain normally can be seen also. Hematoxylin and eosin stain. × 450.

FIG. 3. Liver. The cells in the areas of focal necrosis stained red, demonstrating the presence of pectin. Ruthenium red stain. × 180.



1



2 3

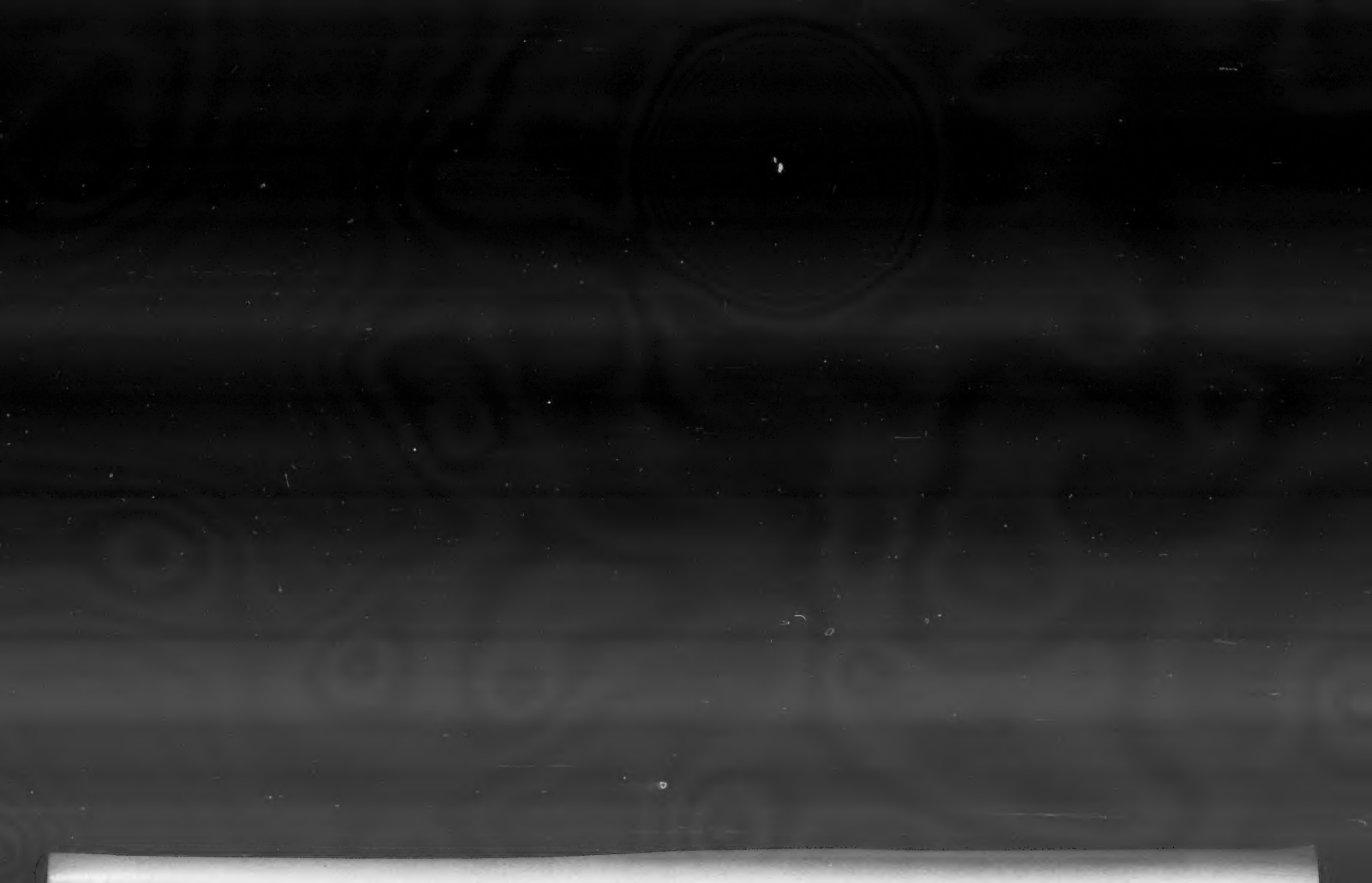
Richter Parenchymatous Lesions Due to Pectin

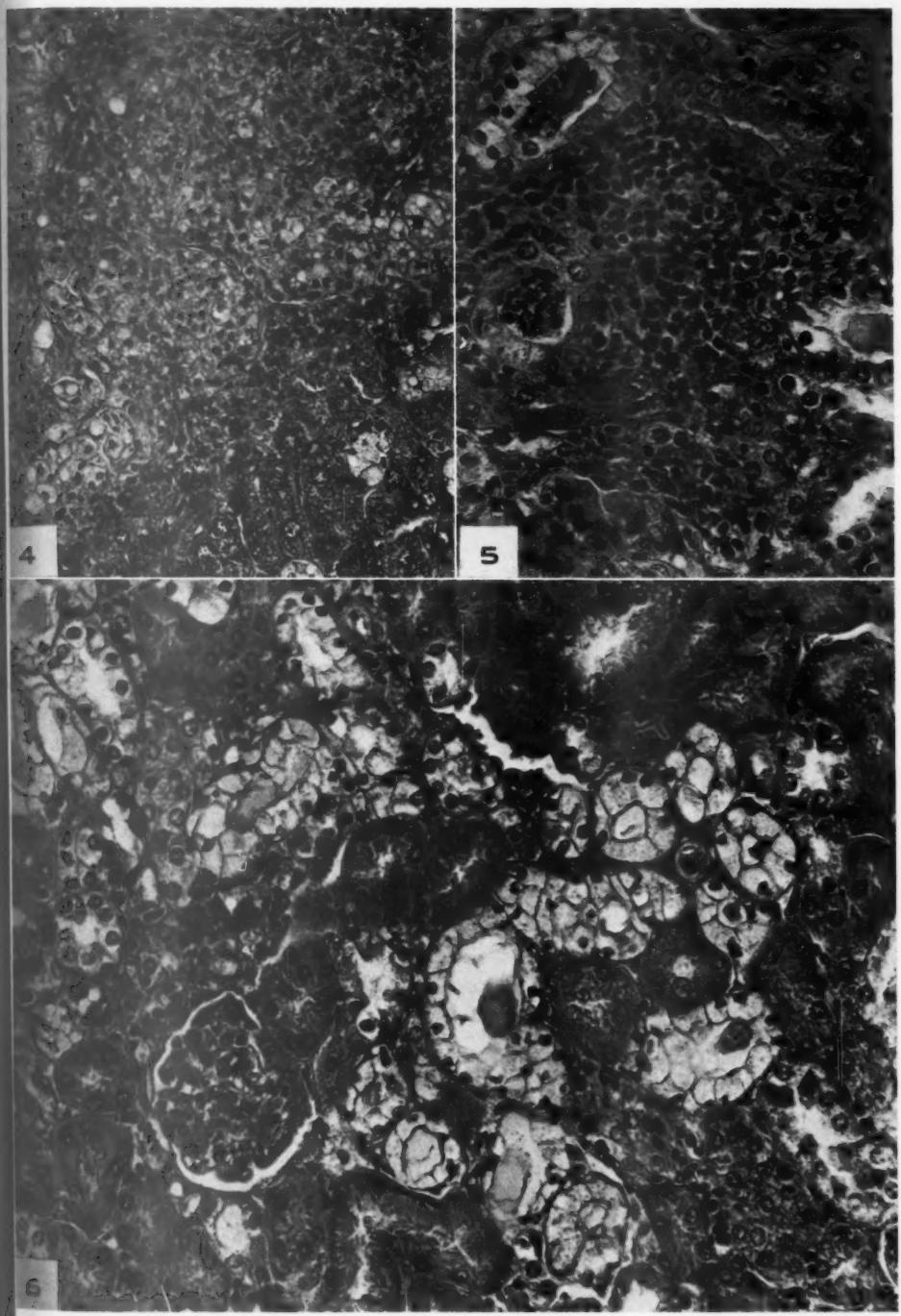
PLATE 61

FIG. 4. Granulation tissue and beginning fibrosis in conglomerated areas of focal necrosis in the liver. Nests of disintegrating foam cells like those in Figure 2 are surrounded and invaded by fibrous tissue. Hematoxylin and eosin stain. × 280.

FIG. 5. Granuloma formation in kidney. There are also three pectin casts in tubules, two of which contain polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 450.

FIG. 6. Kidney. In the tubular epithelium are pale cells, many of which stain grayish blue with hematoxylin and eosin and red with ruthenium red. Nuclei are degenerating. Of note are pectin casts in the lumina of the tubules, some of which have been completely blocked. Hematoxylin and eosin stain. × 450.





Richter Parenchymatous Lesions Due to Pectin

THE EFFECT OF RESTRICTION OF FLUID INTAKE ON THE PRODUCTION OF NEPHROSIS IN RABBITS*

WILLIAM B. WARTMAN, M.D., JAMES M. TUCKER, M.D., and ROBERT B. JENNINGS, M.D.
(From the Department of Pathology, Northwestern University Medical School, Passavant Memorial and Wesley Memorial Hospitals, Chicago, and The Evanston Hospital Association, Evanston, Ill.)

In another paper,[1] experiments have been described which were designed to test the assumption that the presence of previous renal damage might be a factor in the development of bile nephrosis. Using rabbits, one kidney was damaged by occluding the right renal artery and vein for a brief period. At the same time the common bile duct was divided in order to produce jaundice. Under these conditions lesions were produced in the kidneys of about 40 per cent of the animals. The changes occurred chiefly in the cortex and consisted of dilatation of tubules, flattening, degeneration, and necrosis of the lining epithelium, and numerous reddish brown, granular heme casts. These lesions were not accompanied by clinical evidences of renal disease and were not of equal intensity in all animals. They were present in both kidneys, although only one of them had been rendered ischemic. At the time the experiments were performed it seemed possible that the incidence and severity of the lesions might be increased by restricting fluid intake.[2] In order to test this assumption the experiments were repeated using rabbits which had been either partially or completely deprived of water or other fluids for known periods.

EXPERIMENTS

Adult male and female rabbits of mixed laboratory stock were used. They were fed a diet consisting of oats and stale bread which caused the urine to be acid. Thirty-one rabbits were used, divided into the following groups:

Group 1. Group 1 consisted of 4 animals in which the right kidney was rendered ischemic for 18 minutes, and the common bile duct ligated and divided in order to produce jaundice. These animals were given 10 cc. of water per kg. of body weight for 28 days before operation, and afterwards all water was withheld for the next 4 days, when the animals were killed. During the period when the intake of water was restricted the urinary output was normal, but when water was completely withheld it fell to zero.

Group 2. Group 2 consisted of 7 animals which likewise were jaundiced and subjected to ischemia of the right kidney for 18 minutes.

* Received for publication, February 19, 1949.

This group differed from group 1 in that the fluid intake of the animals was only partially reduced. Before operation they were given all the water they would drink, but after operation they were restricted to 15 cc. of water per kg. of body weight for periods varying from 1 to 22 days, when they were sacrificed.

Group 3. Group 3 consisted of 12 animals which were jaundiced, but the renal vessels were not occluded. This group was designed to test the effect of restricted fluid intake in jaundiced animals in the absence of renal ischemia. Ten of the animals were allowed 15 to 20 cc. of water for periods of 3 to 22 days following operation. The other animals (Table II, rabbits 42 and 47) were given 10 cc. of water per kg. of body weight for 11 and 28 days, respectively, before operation, after which all water was withheld for 4 days, when the animals were killed.

Group 4. Group 4 consisted of 2 animals, one of which was allowed 15 cc. and the other 25 cc. of water per kg. of body weight for 7 days. These animals had been subjected to ischemia of the right kidney for 20 minutes but were not jaundiced. This group was a control for the effect of restricted fluid intake plus renal ischemia in the absence of jaundice.

Group 5. Group 5 consisted of 6 animals which were subjected to no operative procedures whatever. They served as a control group in which could be studied the effects of simple dehydration in the absence of jaundice and renal ischemia. Four of the animals (Table III, rabbits 43, 48, 50, and 51) were given 10 cc. of water per kg. of body weight for 28 days, and then all water was withheld for the next 4 days, when they were autopsied. Two other animals were allowed 15 cc. (rabbit 24) and 25 cc. (rabbit 1) of water per kg. of body weight for 10 and 7 days, respectively, at which time they were sacrificed.

Surgical procedures were performed aseptically under intravenous nembutal anesthesia. The common bile duct was exposed and ligated through a single vertical incision in the right upper quadrant. In the animals in which temporary renal ischemia was produced the renal pedicle was isolated through the same incision and the main renal artery and vein were raised on an aneurysm needle and occluded for 20 minutes. The incision was closed with a single row of interrupted silk sutures.

Blood nonprotein nitrogen and creatinine were determined by the methods of Koch and McMeekin, and of Folin, respectively, using samples of whole blood obtained from an ear vein. The plasma icterus

TABLE I

Effect of Restriction of Fluids on Nephrosis Developing in Jaundiced Rabbits Subjected to Temporary, Unilateral, Renal Ischemia

Rabbit no.	Initial weight	Final weight	Survival	Reaction of urine	Casts in urine	Red blood cells in urine	Initial non-protein nitrogen	Final non-protein nitrogen	Initial creatinine	Final creatinine	Terminal jaundice	Autopsy findings: Heme casts	Dilated tubules	Flat epithelium	Tubular necrosis
	(gm.)	(gm.)	(days)	(pH)			(mg. per 100 cc. of serum)								
Group 1, Complete restriction of water															
44	2924	2330	4K	Insufficient urine			58	71	2.3	4.2	+	+++	+++	+++	o
46	2286		4K	Insufficient urine			41	71	1.6	3.4	+	+	++	++	o
49	3659	2559	4K	Insufficient urine			34	107	2.1	8.5	+	+	+	+	o
45	2348	1781	4K	Insufficient urine			53	92			+	o	o	o	o
Group 2, Incomplete restriction of water															
15	1740	1239	9K	4.5	+++	++					++	++++	++++	++++	++++
37	1824	1824	1K	4.5	o	o					o	+++	+++	+++	++
14	3180	1800	22D	4.5	++	++	41	69			186	++	++	++	o
33	1580	1500	3D	6.0	o	o					+	+	+	+	o
2	1668	1668	3D	4.5	o	o					+	o	o	o	o
28	3484	3017	7K	4.5	+	o	41	50			26	o	o	o	o
29	2137	2137	3D	4.5	o	o					+	o	o	o	o

D = Died.
K = Killed.

index was measured colorimetrically with bichromate standards. The reaction of the urine was measured colorimetrically.

Autopsies were done promptly after the animals died or were killed with air embolism. Pieces of liver and of both kidneys were fixed for 24 hours in 10 per cent buffered formalin and Zenker's fluid-formaldehyde solutions. The blocks were embedded in paraffin, and the sections stained with hematoxylin and eosin, Masson's trichrome, McManus' periodic acid,[3] and Lepehne's benzidine stains.[4]

Results

Groups 1 and 2. Groups 1 and 2 consisted of rabbits which received either limited amounts of water or no water at all. Since the results were similar, they will be considered together. From Table I it will be seen that moderate to marked changes (2, 3, or 4 plus) were produced in the kidneys of 5 of the 11 animals. In 4 no lesions whatever were discovered while in 2 the lesions were so slight (1 plus) that it seemed best to disregard them. The renal lesions were similar to those previously described,[1] consisting principally of dilatation of both the proximal and distal segments of the tubules, which were lined with flattened epithelial cells and contained numerous heme casts. These changes were found in both kidneys in spite of the fact that only one of them had been subjected to temporary ischemia. Thus it appeared that withholding water from rabbits neither increased nor decreased the incidence or the severity of the renal lesions.

It is interesting to note that in the animals which were partially dehydrated for 28 days before operation, and then completely deprived of water for 4 days, there was a rise in both the nonprotein nitrogen and creatinine content of the blood (Table I), which was not related to the presence or absence of demonstrable renal lesions. It may, perhaps, be explained by the fact that the animals which were deprived of water were anuric at the time of death.

Group 3. The results obtained in group 3 are shown in Table II. These animals were jaundiced but were not subjected to renal ischemia. Of the 12 rabbits, one had slight renal changes similar to those found in the animals in groups 1 and 2 and which were disregarded because they were so slight. Another one showed pigmented casts but no other lesions. The remaining 10 animals were negative. Thus it appears that, in the absence of temporary, unilateral, renal ischemia, dehydration does not regularly produce renal lesions in jaundiced rabbits.

Group 4. The animals in group 4 were subjected to renal ischemia

TABLE II

TABLE II

Effect of Restriction of Fluids on Jaundiced Rabbits (Group 3)

Rabbit no.	Initial weight	Final weight	Survival	Reaction of urine	Casts in urine	Final nonprotein nitrogen	Terminal jaundice	Autopsy findings: Heme casts	Dilated tubules	Flat epithelium	Tubular necrosis
	(gm.)	(gm.)	(days)	(pH)		(mg./100 cc.)					
7	2528	2083	7K	5.0		57	55	o	o	o	o
8	2062		3D	Acid			+	o	o	o	o
12	1444	1420	4D	Acid		57	+	o	o	o	o
18	2411	2000	10K	5.0	++	71	78	+	+	+	o
22	2189	1440	11K	4.7	++	58	55	o	o	o	o
25	3083	1830	22K	4.5	++	63	43	o	+	o	o
27	3466	3000	11K	5.0	+	50	57	o	o	o	o
32	2103		3D	Acid			+	o	o	o	o
34	1852	1852	3D	Acid			+	o	o	o	o
35	2157		5K	5.0	+	88	46	++	o	o	o
42	2653	1890	4K	Acid		70	+	o	+	o	o
47	2458	2105	4K	Acid		71	+	o	o	o	o

D = Died.
K = Killed.

TABLE III

Effect of Restriction of Fluids on Rabbits with Temporary, Unilateral, Renal Ischemia (Group 4) and Normal Rabbits (Group 5)

Rabbit no.	Initial weight	Final weight	Survival	Reaction of urine	Initial nonprotein nitrogen	Final nonprotein nitrogen	Initial creatinine	Final creatinine	Autopsy findings: Heme casts	Dilated tubules	Flat epithelium	Tubular necrosis
	(gm.)	(gm.)	(days)	(pH)	(mg. per 100 cc. of serum)							
Group 4, Rabbits with temporary renal ischemia												
4	2350	2255	7K	4.5					o	o	o	o
26	1673	1454	7K	4.5					o	o	o	o
Group 5, Normal rabbits												
1	2074	1965	7K	6.0					o	o	o	o
24	1881	1753	10K	4.5					o	o	o	o
43	2505	1573	32K	Acid	58	71	2.3	4.2	o	o	o	o
48	3061	1499	28D	Acid					o	+	o	o
50	2631		29D	Acid	34	34	1.3	1.3	o	+	o	o
51	3000	2782	9D	Acid	39	79	1.7	1.7	o	o	o	o

D = Died.
K = Killed.

and partial dehydration but were not jaundiced. At the end of 7 days no changes were found in the kidneys (Table III).

Group 5. Rabbits in group 5 were neither jaundiced nor subjected to renal ischemia. They were, however, either partially or completely deprived of fluids. As none of the 6 animals in the group showed significant changes in the kidneys, it seems likely that simple restriction of fluid intake does not produce renal lesions in otherwise healthy rabbits (Table III). Chemical determinations on the blood were made in 3 animals. One showed a moderate rise in nonprotein nitrogen and creatinine, and a second animal showed a rise of nonprotein nitrogen but not of creatinine. The third animal showed no increase in nitrogen metabolites.

Discussion

The purpose of these experiments was to determine whether or not dehydration had an effect upon the nephrosis which developed in jaundiced rabbits after they were subjected to temporary, unilateral, renal ischemia. The results indicate that, under the conditions of these experiments, dehydration had no demonstrable effect. It neither enhanced nor prevented the appearance of nephrosis. Thus about 40 per cent of jaundiced rabbits with temporary, unilateral, renal ischemia showed lesions in both kidneys regardless of whether the fluid intake was restricted or altogether cut off. These lesions were not regularly present in dehydrated normal animals, nor in jaundiced rabbits which were dehydrated but not subjected to renal ischemia, nor in rabbits with temporary renal ischemia but without jaundice.

The renal lesions in these rabbits were identical with those which have been described previously.[1] Although the kidneys were bile-stained and the pigment could be seen microscopically, they were not the lesions of bile nephrosis, as that term is ordinarily understood.

The liver was examined microscopically for all animals and no significant abnormalities were discovered. Depending upon the length of survival, there was slight to moderate periportal fibrosis and dilatation of bile ducts, but no cirrhosis. Interpretation of this observation was made difficult by the high incidence of parasitic infection.

Summary

Under the conditions which prevailed in these experiments, prolonged and severe restrictions of fluid intake (dehydration) had no demonstrable influence on the nephrosis which results in jaundiced rabbits from the induction of temporary, unilateral, renal ischemia.

REFERENCES

1. Wartman, W. B., Rusterholz, A. P., and Tucker, J. M. The effect of renal ischemia on the production of experimental nephrosis in jaundiced rabbits. *Am. J. Path.*, 1950, **26**, 235–247.

2. Lalich, J. J. The influence of available fluid on the production of experimental hemoglobinuric nephrosis in rabbits. *J. Exper. Med.*, 1948, **87**, 157–162.

3. McManus, J. F. A. The periodic acid routine applied to the kidney. *Am. J. Path.*, 1948, **24**, 643–653.

4. Herxheimer, G. *Lepehne*sche Färbung des Hämoglobins. In: Abderhalden, E. Handbuch der biologischen Arbeitsmethoden. Urban & Schwarzenberg, Berlin & Wien, 1924, Abt. 8, Teil 1, 1 Hälfte, pp. 236–240.

EXPERIMENTAL TOTAL MIDZONAL HEPATIC NECROSIS*

BERNARD BLACK-SCHAFFER, M.D., DAVID S. JOHNSON, M.D., and
WALTER G. GOBBEL, JR., M.D.
(*From the Department of Pathology, Duke University School of Medicine, Durham, N.C.*)

In the literature available to us we have failed to find reports of total and complete experimental midzonal hepatic necrosis. Himsworth,[1] in his excellent review of laboratory and natural liver disease, discussed midzonal necrosis not as an aspect of experimental pathology but only in relation to human disease. Its rarity is attested by the following quotation:

> "After consulting numerous pathologists, both in Europe and America, I have only been able to see a few specimens for which any claim could be made that they showed a complete midzonal necrosis. In all save one the midzonal appearance was seen only in a few lobules in the section, the remainder showing varying degrees of incomplete midzonal focal necrosis. In a single section, found for me by Professor Arnold Rich of Baltimore, the lesion seemed to affect all lobules. It is apparent, therefore, that a precise midzonal necrosis, if it exists, and is not either an artefact dependent upon vagaries in cutting the section or a transient, intermediate stage in a more extensive lesion, occurs with extreme rarity."

It has fallen to our lot to encounter this lesion, during an investigation of heightened hepatic sensitivity to injury induced by hyperthyroidism.

METHODS

Stock rabbits of both sexes and various breeds were subjected to experimentation in groups of 5, occasionally 10. Hyperthyroidism was produced by the daily administration via stomach tube of 0.71 gm. of desiccated thyroid powder suspended in tap water, until the animals lost approximately 25 per cent of their original weight. Twenty-four hours after the last dose of thyroid substance the animals were given a subcutaneous injection of 0.1 cc. of chloroform per kg. of body weight, diluted in mineral oil. They were sacrificed 24 hours later either by air embolism, rabbit punch, or both, immediately autopsied, and organ sections fixed in 10 per cent aqueous formaldehyde (fat stains) and Helly's modified Zenker's fluid. In addition, specimens of many of the livers were placed immediately in absolute alcohol for glycogen studies.

EXPERIMENTS AND RESULTS

It was determined in a series of experiments (Table I) that normal animals weighing between 2100 and 2900 gm. responded to subcuta-

* Presented at the Forty-sixth Annual Meeting of The American Association of Pathologists and Bacteriologists, Boston, April 15, 1949.
Received for publication, June 27, 1949.

neously administered chloroform in doses as small as 0.1 cc. per kg. with minimal central necrosis. When 0.05 cc. of chloroform per kg. or less was given, no damage was demonstrable. When the dosage was increased over 0.1 cc. per kg., the injury was more extensive but of the same quality, and in the same central location. The 10 animals receiving the 0.1 cc. per kg. dose showed no clinical evidence of disease 24 hours after injection. The livers upon gross examination revealed no recognizable lesions.

The necrosis, as illustrated in Figure 1, was central, and consisted

TABLE I

Determination of Dosage of Chloroform for the Production of Central Hepatic Necrosis in Normal Rabbits

Experiment no.	No. of rabbits	$CHCl_3$/Kg.	No. with central necrosis
1	5	0.2 cc.	4
2	10	0.1 cc.	5
3	10	0.05 cc.	0

in the appearance of fat vacuoles of varying size within some of the cells, hyaline necrosis of others, with fragmentation or pyknosis of nuclei and the presence of a small number of polymorphonuclear leukocytes.

Since numerous investigators have demonstrated the heightened sensitivity of the liver of thyroid-fed cachectic animals to various noxae—infection,[2] hypoxemia,[3] halogenated hydrocarbons[4]—the reaction of rabbits given desiccated thyroid gland over a period sufficiently long to result in a loss of 25 per cent of original body weight was tested. Eight rabbits were given thyroid substance (0.71 gm. per diem) until approximately 25 per cent of the original body weight had been lost, and were then sacrificed. The livers were alike in that none showed necrosis. All showed those changes long known to characterize starvation: loss of glycogen and decrease in size of hepatic cells with dilatation of sinusoids (Fig. 3).

Five groups of 5 animals each were established (Table II). Two groups (A, B) received, in addition to desiccated thyroid gland, food (oats and alfalfa pellets) *ad libitum*, and 2 (C, D) were starved. The fifth group (E), while likewise starved, received thyroid gland in daily doses. All groups had water at all times. One group of the two pairs (B and D) received subcutaneous daily doses of vitamins B and C.*

The results were unexpected. In group A (Table II), receiving food

* Thiamine hydrochloride, 4 mg.; riboflavin, 1.2 mg.; niacinamide, 8 mg.; pyridoxine hydrochloride, 1.2 mg.; sodium pantothenate, 1.2 mg.; ascorbic acid, 20 mg.

but no vitamins, all animals revealed what has since been recognized as typical midzonal necrosis (Figs. 4, 5, 6, and 7). Numerous sections from different lobes revealed a constant appearance, namely, fatty change of a narrow zone of liver lying between the portal and central areas. The band wound its way between the portal fields and central and hepatic veins. When cut at right angles to a central vein it appeared as a circle or oval, completely enclosing the central zone and vein. It contained numerous necrotic cells showing hyalinization of cytoplasm, karyorrhexis and karyolysis, early polymorphonuclear leukocytic infiltration, and accumulation of fat. Individual cells in the portal or central areas might likewise show necrosis.

TABLE II

Distribution of Hepatic Necrosis Following Injection of Chloroform in Rabbits under Certain Controlled Dietary Conditions

Rabbit no.	Fed		Starved		
	A Thyroid gland without vitamins	B Thyroid gland with vitamins	C Without vitamins	D With vitamins	E Thyroid gland without vitamins
1	Midzone	Midzone	Extensive	Central	Midzone
2	Midzone	Central*	Central	Central	Midzone
3	Midzone	†	Central	Central	Midzone
4	Midzone	‡	Central	Extensive	Central
5	Midzone	‡	Central	Extensive	Central

* Pneumonia, no chloroform injected.
† Lost only 18 per cent body weight after 14 days, no necrosis.
‡ No chloroform injected, no necrosis.

Of the 5 animals in group B (Table II), given daily vitamin supplements in addition to food *ad libitum*, one showed midzonal necrosis, and one central necrosis.

The latter died unexpectedly on the 11th day of the experiment, having lost approximately 22 per cent of his original body weight (2,755 gm.). Autopsy revealed necrotizing pneumonia, probably due to the accidental instillation of thyroid suspension into the trachea.

A third animal had lost only 18 per cent of his original body weight after 14 days of thyroid administration. Its liver, after chloroform, revealed loss of glycogen with no necrosis, characteristic of starvation without additional injury such as chloroform or infection.

The fourth rabbit lost weight rapidly immediately after the first thyroid dose, by the seventh day had lost approximately 28 per cent of his body weight, and died, as far as could be determined, from starvation. The difference in the reactions of the last 2 rabbits, whose initial weights (2790 gm.) were identical, to thyroid, represents the extremes sometimes encountered. Those animals who rapidly lost weight stopped eating with or, as will later be demonstrated, without accessory vitamins.

The fifth subject died from causes unknown on its fourth day, having lost very little weight.

The work of Miller and Whipple[5] established that protein depletion of the liver is the chief cause of hypersensitivity to noxious agents. They, along with others (notably Drill[6]) showed that hyperthyroidism accelerates the process of protein depletion by interfering with appetite and thus with the intake of calories and vitamins, and by increasing the rate of utilization of the two. Because of this it was determined to test the effect of a minimal toxic dose of chloroform (0.1 cc./kg.) upon rabbits starved with and without vitamins B and C (Table II).

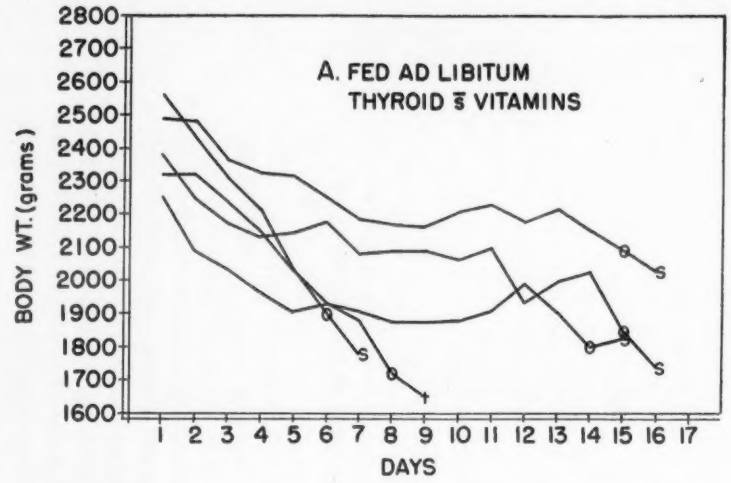
Of the 5 animals receiving only water *ad libitum* (C), one showed massive necrosis, and the remaining 4 central hepatic necrosis of varying degree. The least was far more severe than the most pronounced among the normal animals of Table I.

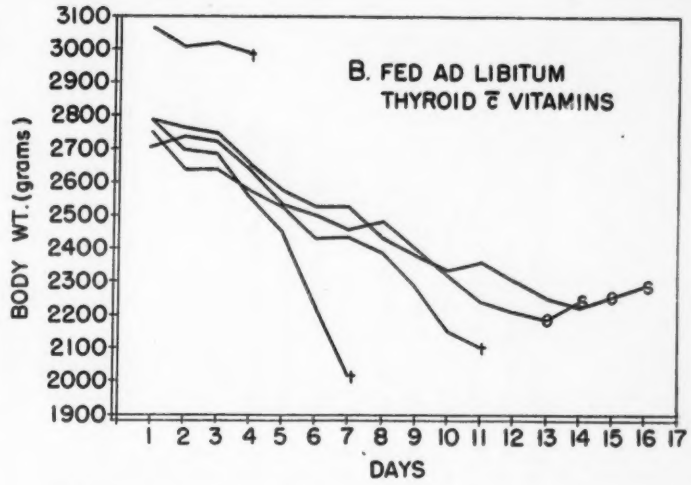
The starved rabbits (D), receiving the same dosage of vitamins B and C as group B, showed by far the most devastating necrosis of all. In 2 instances almost all of the individual liver lobules were involved. That the lesion probably began with central necrosis is intimated by the survival of some periportal cells and the presence of a clear-cut central necrosis in 3.

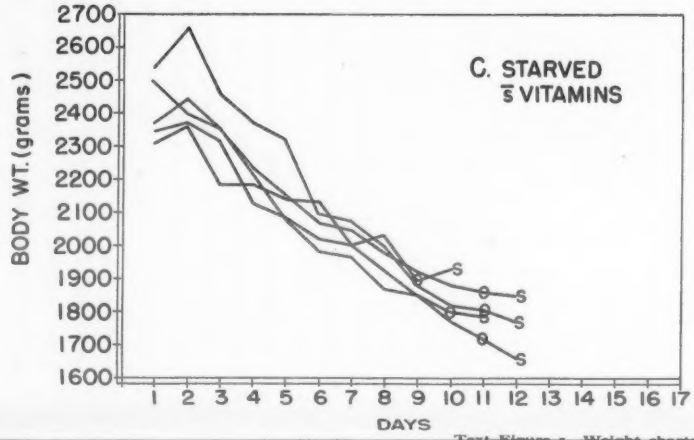
A further experiment (Table II, E) consisted in combining daily thyroid feedings and starvation (water *ad libitum*) for a period of 4 days, then, after a 24-hour interval, the subcutaneous administration of chloroform followed by sacrifice 24 hours later. Three of the 5 rabbits showed characteristic midzonal necrosis. In 2 of the 3, the lesion was histologically farther advanced than hitherto seen but still spared the central and periportal zones. The remaining 2 animals showed characteristic central hepatic necrosis. As illustrated in Text-Figure 1, B, the administration of large doses of thiamine as well as other factors of vitamin B and ascorbic acid had no appreciable effect upon the progressive weight loss of rabbits as compared to the animals of group A.

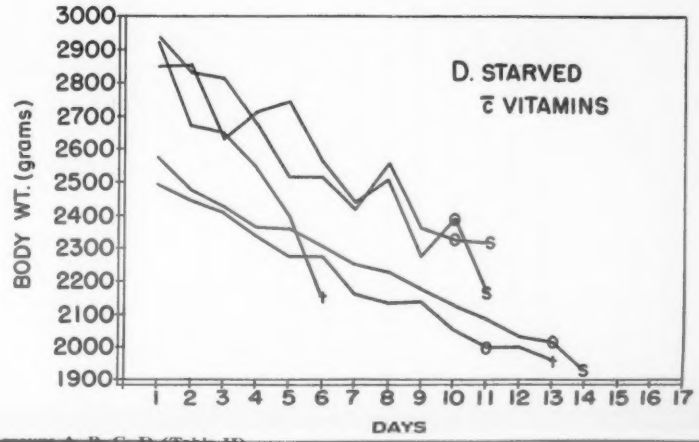
In both groups, A (no vitamin supplements) and B, some individuals, immediately after the first dose of thyroid gland, lost their appetites and proceeded precipitously to lose weight, despite large supplements of thiamine (4 mg. daily). Hyperthyroid dogs[7,8] and rats,[9] in contrast, are able to maintain their appetite and weight more effectively when thiamine is added to their diets. Thus, in this respect, the rabbit differs from the dog and rat.

Weight charts C and D (Text-Fig. 1) delineate the course of rabbits starved without and with vitamin supplements. In these groups no







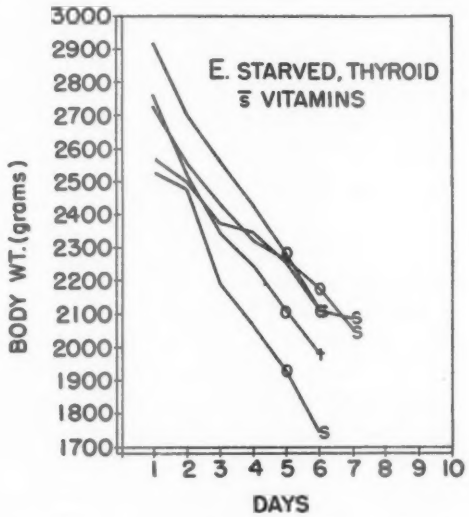


Text Figure 2. Weight charts of groups A, B, C, D (Table II).

differences were expected or found. Of interest, however, is the fact that the rate of weight loss in these small groups (hyperthyroid A, B, and simple starvation C, D) does not appear to vary appreciably. Yet a qualitative difference is indicated by the production of midzonal hepatic necrosis in the one and not in the other. This is true despite the amplified sensitivity to noxae of the livers in all animals.

Of further interest is the one rabbit of group D who proceeded to lose weight rapidly, as did some of the animals in the hyperthyroid groups A and B, indicating the wide degree of individual variation to starvation.

Text-Figure 2 depicts the weight loss of rabbits which were both starved and given thyroid. All responded as did the individuals of



Text-Figure 2. Weight chart of group E (Table II).

A, B, and D of the preceding paragraph. The increased rate of weight loss as compared to C and D clearly indicates the accelerating influence of hyperthyroidism.

Discussion

The various possibilities in the pathogeneses of zonal hepatic necrosis have been reviewed by Himsworth,[1] with adequate citation of the literature. Glynn and Himsworth[10] demonstrated the dependence upon the lobular blood supply. Thus periportal necrosis is produced by the direct effect of lethal cytotoxins in the periportal region where they first engage the liver cells. If the toxin is non-lethal by nature or because of dilution,

it may cause swelling of the periportal cells with a decrease in the caliber of the sinusoids, thus interfering with the centripedal flow of blood from portal field to central vein. If the flow is sufficiently impaired, the central zone cells, the most distant, would be first damaged because of the ensuing greater hypoxemia. The quality of the damage may vary from fatty change to necrosis. Thus the identity of the etiologic agent in zonal hepatic necrosis is of secondary importance to the site of its effect. In this manner Glynn and Himsworth have explained the common end-result of injury by arsenic, chloroform, and carbon tetrachloride, namely, central necrosis. The thesis is an attractive one and their evidence, India ink injections of the portal circulation, convincing. This explanation of zonal necrosis appeared satisfactory until the midzonal necrosis illustrated and described above was discovered. Certainly, in view of the similarity of the livers of the rabbit and the rat (the animals most extensively used in liver experiments) and their similarity in turn to the hepatic circulation and architecture of other mammals, it would appear that true and complete midzonal necrosis could not be explained on the basis of the lobular circulation, since the most highly vulnerable area, the central zone, remains intact.

In the past, numerous workers[11] have suggested that the various zones of the hepatic lobule perform specific functions. These differences were believed to account, at least in part, for selective zonal susceptibility to various toxins. Unfortunately, solid evidence in support of this concept has been wanting. Despite this, it is suggested that midzonal necrosis as produced in the above experiments may be best explained by selective function of that zone.

It is now common knowledge that simple severe starvation, without infection or intoxication, produces no necrosis of the liver cells but only loss of glycogen and depletion of protein. This condition, however, results in increased sensitivity of the liver to toxic agents such as the halogenated hydrocarbons. However, the injury is characteristically central (Table II, C and D). Starvation as measured by body-weight loss induced by experimental hyperthyroidism, without complicating infection or intoxication, elicits the same response from the liver: loss of glycogen, protein depletion, and increased susceptibility to chloroform but no necrosis. However, when chloroform in small doses is given to such an animal, instead of central necrosis, total midzonal necrosis may appear (9 of 15 animals).

If rabbits (E) are placed upon a regime of complete starvation and daily thyroid substance, 4 doses are sufficient to establish the conditions necessary for the production of midzonal necrosis by chloroform

injection. The only noticeable difference between this group and the others is the very rapid depletion of weight (Text-Figs. 1 and 2). (No attempt has been made to determine the minimal interval necessary to establish the prerequisites for midzonal necrosis.)

It is apparent, therefore, that starvation alone will not produce the hepatic changes conditioning midzonal necrosis. Nor, for that matter, will chloroform alone result in such a change. Starvation, however, influenced by large doses of thyroid substance does achieve that end. It is suggested, consequently, that the metabolism of the midzone is altered quantitatively and/or qualitatively. This alteration creates a sufficient functional differential from the periportal or central zones, so that the midzone responds to chloroform with generalized necrosis, in contrast to the relatively minor degree of damage exhibited by the other two zones.

In addition to the above, a further observation is pertinent to the discussion. In the course of the experiments 6 animals developed "aspiration" pneumonia due to accidental intubation of the trachea instead of the esophagus. All 6 were receiving thyroid substance and losing weight; all died before conclusion of the experiment and showed at autopsy thyroid cachexia, pneumonia, and central hepatic necrosis. While no attempt has been made to investigate this lead systematically, the fact remains that all rabbits whose liver susceptibility to toxins was increased by thyroid cachexia, and who developed pneumonia, presented not midzonal but central necrosis of the liver. Sealy[2] demonstrated that rabbits made cachectic by thyroid substance regularly developed central hepatic necrosis when cutaneous suppuration was induced. This observation is now extended to include pneumonia.

If all noxae causing central hepatic necrosis accomplish that lesion via curtailment of the intralobular circulation (Glynn and Himsworth[10]), the question arises: why does chloroform, a classic producer of central necrosis, elicit midzonal necrosis in a large number of thyroid-cachectic rabbits while in similarly prepared animals infection results in central necrosis? Very possibly some toxins may produce zonal necrosis via the mechanism brilliantly demonstrated by Glynn and Himsworth. Yet it appears that the nature of the toxic agent and the functional status of the lobular zones may likewise dictate the site of hepatic necrosis.

Exactly why or how the thyroid affects the midzone is not known. Elucidation has not been attempted in the above experiments. However, the method used represents a potential tool toward a better understanding of the function of the liver lobule, since, for the first time, a

reliable method of producing true generalized midzonal necrosis is made available.

Conclusions

Total midzonal necrosis of the liver may be produced in hyperthyroid rabbits, who have lost approximately 25 per cent of their body weight, upon subcutaneous injection of chloroform in a dose of 0.1 cc. per kg.

Starvation, with or without large thiamine supplements, to a similar level or weight loss, increases the susceptibility of the liver to chloroform with the production of characteristic central necrosis.

Normal rabbits likewise respond to larger doses of chloroform with characteristic central necrosis.

Thyroid-cachectic rabbits respond to pneumonia with central hepatic necrosis.

It is proposed that the hyperthyroid state selectively affects the hepatic midzone qualitatively or quantitatively so as to amplify its sensitivity to chloroform beyond that of the periportal or central zones.

The response of thyroid-cachectic rabbits to chloroform (midzonal necrosis) and to infection (central necrosis) reaffirms the qualitative importance of the toxic agents in the production of zonal necrosis.

REFERENCES

1. Himsworth, H. P. The Liver and Its Diseases. Harvard University Press, Cambridge, Mass., 1947, 204 pp.
2. Sealy, W. C. Rôle of infection in the pathogenesis of liver necrosis in hyperthyroidism. *Ann. Surg.*, 1942, **116**, 851–859.
3. McIver, M. A., and Winter, E. A. Deleterious effects of anoxia on the liver of the hyperthyroid animal. *Arch. Surg.*, 1943, **46**, 171–185.
4. Goodpasture, E. W. The influence of thyroid products on the production of myocardial necrosis. *J. Exper. Med.*, 1921, **34**, 407–423.
5. Miller, L. L., and Whipple, G. H. Chloroform liver injury increases as protein stores decrease. *Am. J. M. Sc.*, 1940, **199**, 204–216.
6. Drill, V. A. Interrelations between thyroid function and vitamin metabolism. *Physiol. Rev.*, 1943, **23**, 355–379.
7. Himwich, H. E., Goldfarb, W., and Cowgill, G. R. Studies in the physiology of vitamins. XVII. The effect of thyroid administration upon the anoxia characteristic of lack of undifferentiated vitamin B. *Am. J. Physiol.*, 1931–32, **99**, 689–695.
8. Drill, V. A., Shaffer, C. B., and Overman, R. Liver function, pulse rate and temperature of hyperthyroid dogs. *Am. J. Physiol.*, 1942–43, **138**, 370–377.
9. Drill, V. A. Effect of vitamin B_1 and vitamin B_2 complex on the loss of weight produced in rats by experimental hyperthyroidism. *Proc. Soc. Exper. Biol. & Med.*, 1938, **39**, 313–316.
10. Glynn, L. E., and Himsworth, H. P. The intralobular circulation in acute liver injury by carbon tetrachloride. *Clin. Sc.*, 1948, **6**, 235–245.
11. Cowdry, E. V. Special Cytology. Paul B. Hoeber, Inc., New York, 1928, p. 230.

[*Illustrations follow*]

DESCRIPTION OF PLATES

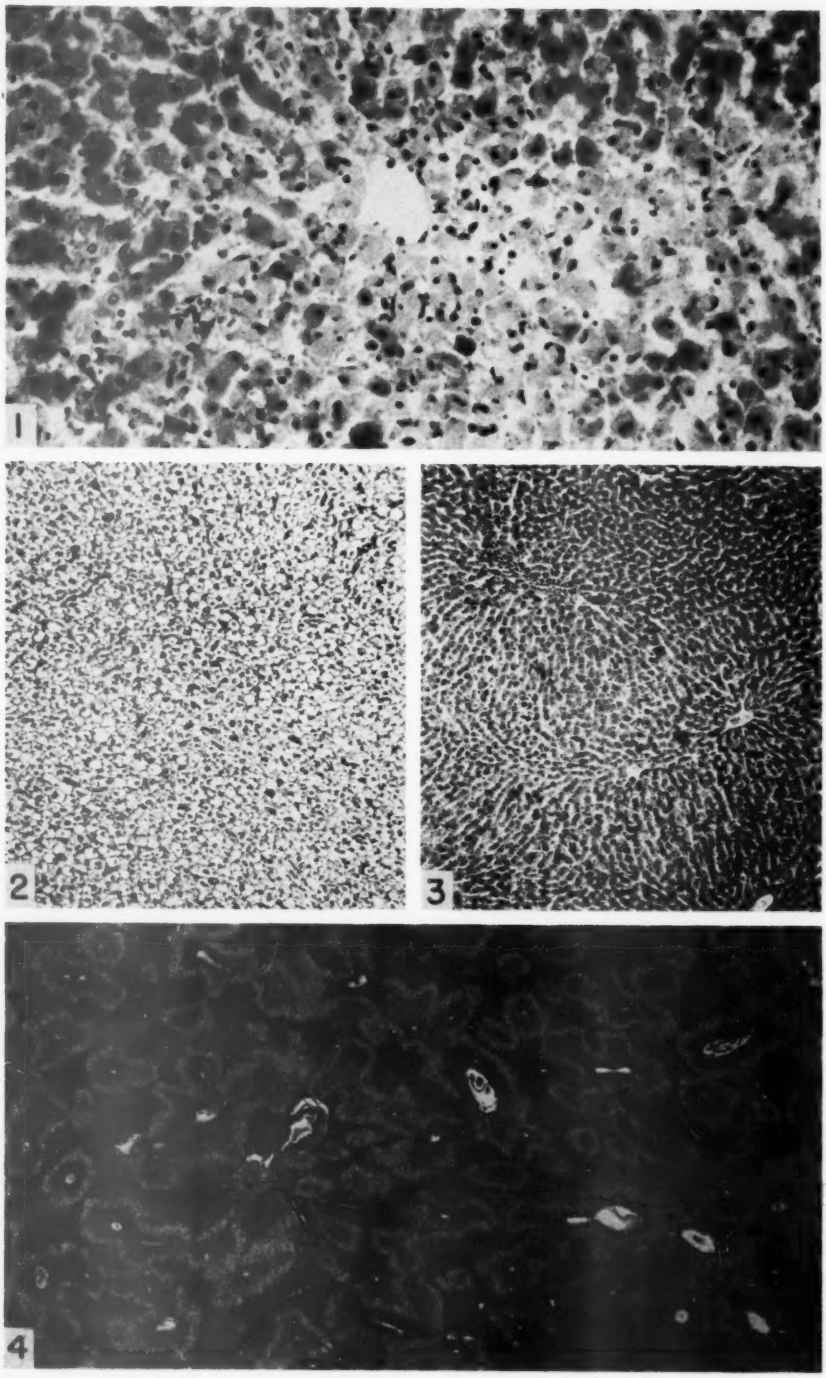
PLATE 62

FIG. 1. Minimal central necrosis of liver in a normal rabbit treated with a subcutaneous injection of 0.1 cc. of chloroform per kg. Animal sacrificed 24 hours after injection.

FIG. 2. The appearance of normal rabbit liver. Cells are distended with glycogen and the sinusoids visible only by virtue of acute passive congestion.

FIG. 3. The liver of a starved rabbit showing loss of glycogen and prominent sinusoids.

FIG. 4. The midzones are the meandering, circular to oval, pale bands enclosing central and hepatic veins and picketed by the dark portal fields.



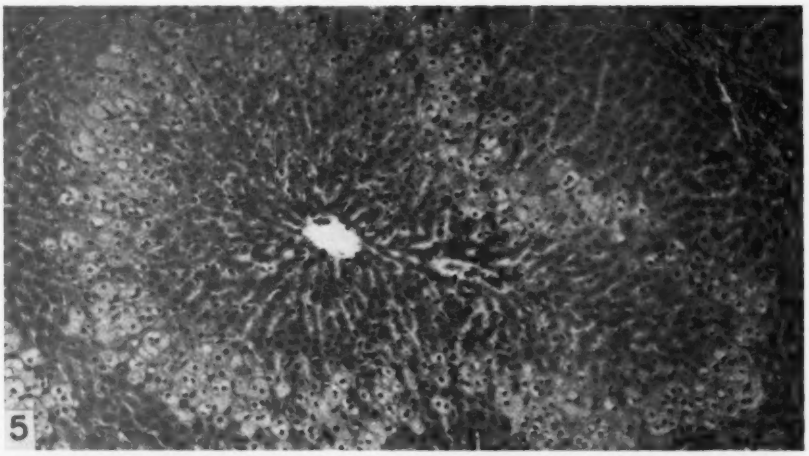
Black-Schaffer, Johnson, and Gobbel Midzonal Hepatic Necrosis

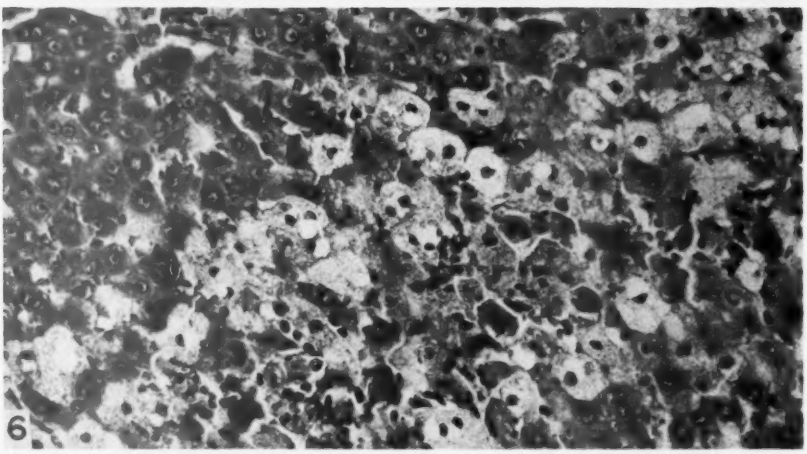
PLATE 63

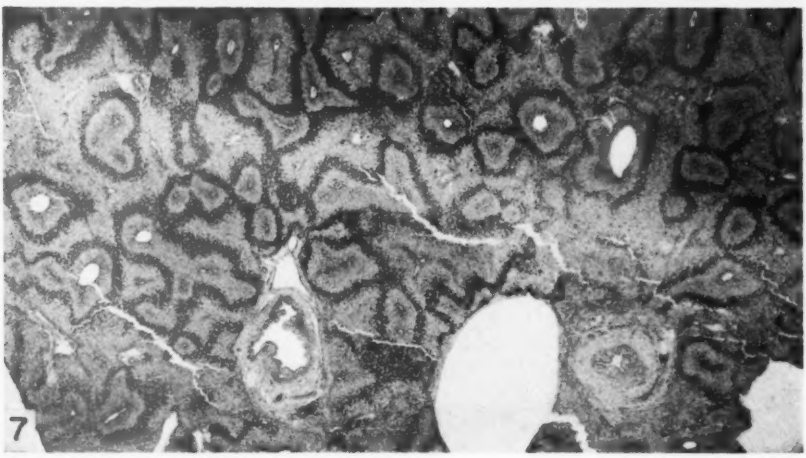
FIG. 5. Fatty and necrotic midzone shown in relation to its central vein and one of its portal fields.

FIG. 6. A higher power view of the midzone revealing the necrosis, lipidic accumulation, and moderate leukocytic exudate.

FIG. 7. Sudan IV preparation showing the degree of the process and the limitation of the accumulated fat to the midzones.







Black-Schaffer, Johnson, and Gobbel Midzonal Hepatic Necrosis

INTRA-UTERINE RESPIRATION-LIKE MOVEMENTS IN RELATION TO DEVELOPMENT OF THE FETAL VASCULAR SYSTEM

A Discussion of Intra-uterine Physiology Based upon Cases of Congenital Absence of the Trachea, Abnormal Vascular Development, and Other Anomalies *

George Milles, M.D., and Dennis B. Dorsey, M.D.†

(From the Pathology Laboratories, Augustana Hospital, and Department of Pathology, University of Illinois, College of Medicine, Chicago, Ill.)

Clinical observation, and the experimental work of Snyder and Rosenfeld,[1-3] Barcroft and Barron,[4] and others,[5-7] have established the fact that the fetus makes respiration-like movements in utero. These studies and, more especially, those of Davis and Potter,[8] indicate that such movements are a normal part of fetal activity, and that intra-uterine respiratory movements normally result in the aspiration of amniotic fluid into the fetal lung. Whether or not intra-uterine respiration is a vital function has not been established.

If intra-uterine respiratory activity is necessary for the normal development or survival of the fetus, interference with it should result in abnormal development or death. If it can be shown that interference with intra-uterine pulmonary expansion, and that alone, produces maldevelopment of some organ or system, it will constitute proof that intra-uterine respiratory movements are physiologic. We were unable to find a report of any anomaly that could be shown to result only from interference with intra-uterine pulmonary expansion.

Material

This report is based upon necropsies performed in cases of prenatal and neo-natal deaths in the Augustana Hospital, Chicago. The first 2 cases reported in detail were those of infants who died at birth. Both were victims of anomalies that made respiration impossible. Although the anomalies affecting the respiratory system were dissimilar, the same altered ratio between the third portion of the aortic arch and the ductus arteriosus was present in both infants. The third case was that of a stillborn infant who had a pulmonary anomaly that, in itself, would not prevent respiration but as an anencephalic monster probably lacked central nervous system control for respiratory movement.[9] No vascular anomaly was found in this case.

* Received for publication, February 23, 1949.

† Now at Pathology Laboratories, Lakeview Hospital, Danville, Ill.

Report of Cases

Case 1

Case 1 was a female infant born on the service of Dr. William Browne. The mother's past history was negative, and the pregnancy, her first, had been without incident. Fetal heart tones were normal throughout labor. Delivery was completed by low forceps. The child appeared normal except that the cardiac impulse was displaced to the right side of the chest. The infant made numerous unsuccessful attempts to breathe. A tracheal catheter was introduced, but even with the catheter in place it was impossible to force air into the lungs.

At autopsy, the body measured 52 cm. from crown to heel. It weighed 3572 gm. and was normally developed and well nourished. The head and upper extremities were cyanotic.

The left diaphragm was absent except for a narrow shelf of muscle which extended onto the anterior surface of the esophagus to which it was attached. Distal to this attachment the esophagus bent sharply upwards and to the left, forming a complete loop. The stomach and spleen were located behind the heart in a hernial sac formed by the mediastinal pleura projecting into the right pleural cavity. The left pleural cavity contained most of the small and large bowel and the left lobe of the liver. The trachea deviated to the right and was patent. The right lung appeared normally developed, but was non-crepitant and firm. The left lung was markedly hypoplastic, and resembled the lung in a similar case reported by Potter and Bohlender.[10]

The heart was displaced to the right, with the apex at about the midsternum. The cardiac chambers were normally formed and situated. The foramen ovale was anatomically patent. The great vessels arose normally. The ascending aorta was 7 mm. in diameter, but after giving off the usual three branches from the arch, the third portion of the arch was only 5 mm. in diameter. The ductus arteriosus was 8 mm. in diameter, 12 mm. in length, and was patent. The descending aorta was 10 mm. in diameter. The ductus arteriosus exceeded the diameter of both the ascending aorta and the third portion of the arch, the latter by a ratio of 8 to 5 ($I/D^* = 0.62$). This child was unable to breathe after birth by reason of the absence of the diaphragm, which made it impossible to produce a negative pressure in the chest cavity. It is reasonable to suppose that intra-uterine respiration also was impossible.

Potter[11] has observed elongation and narrowing of the aortic isthmus in cases of diaphragmatic hernia in neonatal autopsies. No measurements of the vessels were recorded. According to Potter and Bohlender,[10] a fetal lung that fails to develop grossly as a result of external pressure

* Diameter of the isthmus : diameter of the ductus arteriosus.

from herniated abdominal contents or other causes is also retarded in its histologic development. It exhibits alveolar and vascular growth normal to its size, rather than to the chronologic age of the fetus.

The embryogenesis of the diaphragm is an interesting and complicated process, but since it is of incidental interest here, discussion of it will be omitted. An excellent article on this subject is presented by Bremer.[12]

Case 2

Case 2 was a male infant born at term on the service of Dr. Crean. The mother's past history was negative, and this, her first pregnancy, had been without incident. Fetal heart tones were of good quality and remained so throughout the uneventful labor. Delivery was spontaneous. The infant's heart rate and rhythm were normal, but he made only occasional attempts to breathe, and it was noted that the chest failed to expand with respiratory efforts. Repeated attempts to pass a tracheal catheter were unsuccessful. The child became progressively more cyanotic and died in 20 minutes.

At autopsy, the body was that of an apparently well developed white male infant, 48 cm. in length and weighing 3226 gm. The skin was pale, and no external abnormality was found except bilateral cryptorchidism.

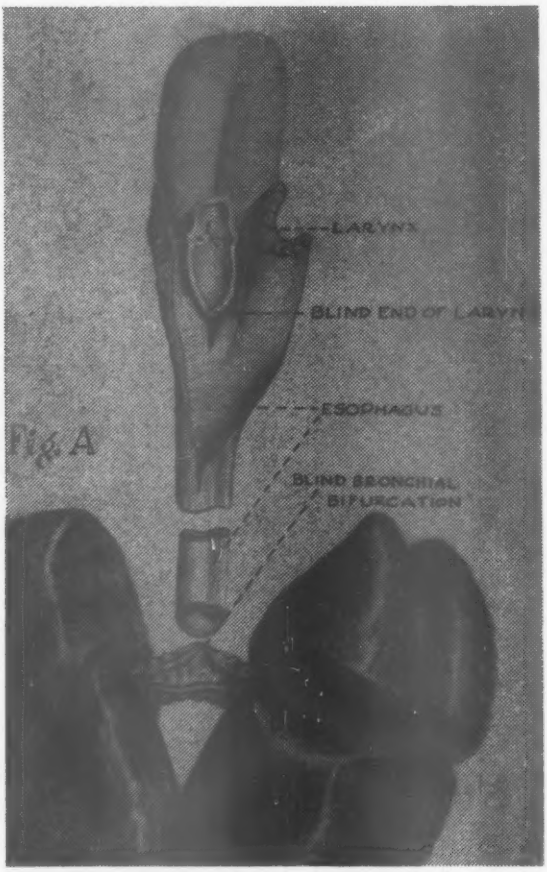
The chest cavity contained a small amount of straw-colored fluid. The lungs were atelectatic and dark purplish red. The esophagus was patent and revealed no fistulous opening. The larynx was normally formed, but ended as a blind pouch at the lower border of the cricoid cartilage. The trachea was absent. The bronchi arose from a blind bifurcation from which point distally they were normally distributed (Text-Fig. A).

The heart was normal in size and outline, and the chambers were in normal relation. The interventricular septum was intact. The foramen ovale was anatomically patent. The pulmonary artery arose from the right ventricle, and after giving off the right and left pulmonary branches, continued as the large ductus arteriosus, which appeared to form the arch of the aorta. The aorta arose from the left ventricle, and gave off the usual three branches from the arch. Beyond the origin of the left subclavian artery the aorta continued as a very narrow vessel into the arch formed by the junction of the ductus arteriosus with the descending aorta. The ductus was at least twice the diameter of the isthmus (I/D = 0.5 or less) (Text-Fig. B).

The only other gross finding of note was the presence of both testicles in the pelvis.

Microscopic study of the lungs revealed marked edema of the interlobular septa. The bronchioles were distended and empty. The alveoli were more or less distended and contained red blood cells. The lining cells were inconspicuous. There was complete absence of aspirated

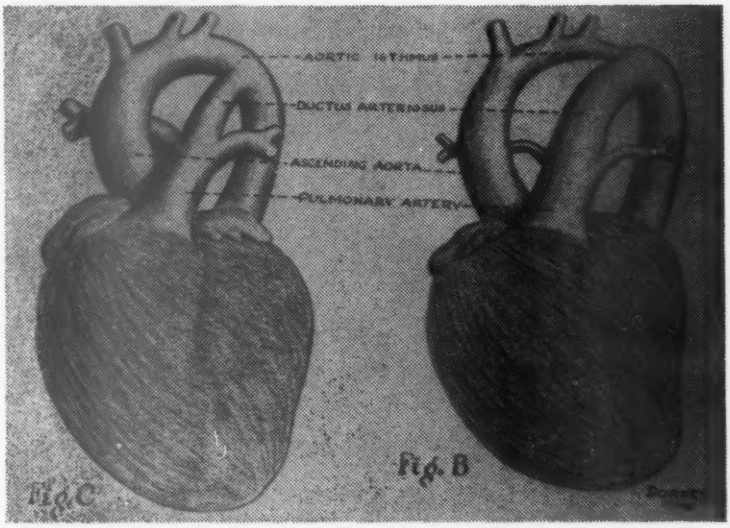
material. The alveoli were well formed, which bears out the observation of Potter and Bohlender[10] in their 2 cases of congenital malformations with no communication between the lung and upper air passages. The vessels were engorged. Microscopic study of transverse sections through the mediastinum at various levels failed to reveal any structure resembling tracheal tissue.



Text-Fig. A. Semi-diagrammatic representation of the tracheal defect in case 2.

Intra-uterine pulmonary expansion was obviously impossible, because there was no communication between the lungs and the larynx. The third portion of the aortic arch was less than half the diameter of the ductus arteriosus.

This case represents an extremely rare anomaly. Marek's case[13] had a fistula connecting the bronchial bifurcation with the esophagus. Fritz,[14] Walcher,[15] and others have reported cases in the foreign literature with fistulae between the esophagus and bronchial tree, combined with an absent, or partially absent, trachea. Payne[16] reported the only instance of absent trachea without communication to the esophagus to be found in the literature. Potter[11] studied a similar case. In neither instance were the great vessels measured.



Text-Figs. B and C. Semi-diagramatic comparison of normal isthmus/ductus size ratio (C) and abnormal ratio observed in cases 1 and 2 (B).

The anlage of the respiratory system appears in the human embryo of 23 segments (3.2 mm.) as a hollow, linear evagination of the ventral side of the primitive gut.[17] The more caudal portion appears first and forms an unpaired, pouch-like termination of the groove, the lung anlage. In normal development a constriction forms at the most caudal portion of the groove and progresses forward, the lateral borders of the groove fusing to form a septum between the tube of the primitive gut and the laryngotracheal tube with its terminal pouch.*

* Patten's[18] description of the development of the trachea and lungs suggests that the entire structure originates as an evagination of the caudal portion of the pharynx. However, the difference is probably one of terminology. The normally developed larynx in this case and in Payne's case[16] suggests that the proximal portion of the tract arises more or less independent of the trachea and the lung. Possibly in these cases separate evaginations appeared for the larynx and lung anlages with no connecting tracheal groove.

The more common anomalies of the trachea and esophagus include a fistula between the two passages with more or less complete failure of development of either the esophagus or trachea. They are explained on the basis of incomplete fusion of the margins of the primitive groove, and on differences in growth rates of the trachea and esophagus during subsequent development (Gruenwald[19]).

It seems logical to assume that the embryogenic defect in this case was a failure of the tracheal groove to form cephalad to the lung anlage, so that when constriction occurred the lung primordium was pinched off in the normal manner but left without communication to the pharynx.

Case 3

Case 3 was a female anencephalic monster, stillborn on the service of Dr. Swenson on June 6, 1947. The history of the pregnancy indicated that the infant was post-mature. Heart tones were last heard on May 7, 1947. Labor was induced, and delivery was spontaneous.

At autopsy, the body was that of a female anencephalic monster with complete spina bifida and a moderate omphalocele. The body weighed 950 gm. and measured 27 cm. from orbit to heel. There was beginning maceration.

The thoracic cavity was markedly diminished by the high position of the intact diaphragm. The thymus was broad, extending laterally into both pleural cavities, and increased in thickness, thus further diminishing the space available for the lungs. The lungs together weighed 3.3 gm. The left lung retained its early fetal configuration. The cut surfaces were firm and pink, speckled with gray. The trachea and main bronchi were patent and empty. The lung tissue did not float.

The heart weighed 5.6 gm. It was normally situated. The four cardiac chambers were normally formed. The interventricular septum was intact. The foramen ovale was anatomically patent. The great vessels arose normally. The ascending aorta was 8 mm. in diameter; the pulmonary artery and the descending aorta were each 7 mm. in diameter. The third portion of the aortic arch was 6 mm. in diameter and 2 mm. in length, and the ductus arteriosus was 5 mm. in diameter and 8 mm. in length.

The adrenals were markedly hypoplastic, and together weighed 0.5 gm.

The lungs appeared to be more hypoplastic than would be expected from the size of the body. The absence of the major portion of the head distorted both the weight and body length, so that these measurements did not serve as an accurate index of the stage of development.

To determine whether the lungs were more hypoplastic than the other organs, we computed the percentage of normal development that each of several organs had attained, with the results shown in Table I. It is evident that the lungs and adrenals were relatively less developed than the other organs. The failure of development of the lungs may be accounted for by the limitations of space in the pleural cavities as a result

TABLE I

Organ	Normal weight at term
	%
Thymus	39.3
Liver	39.0
Kidneys	37.7
Heart	27.3
Spleen	25.4
Adrenals	6.6
Lungs	5.7

of the high diaphragm and the transverse position of the thymus, or perhaps by primary failure of the lungs to develop, as is apparently true of the adrenals in anencephalic monsters.

In microscopic sections of the lungs the alveoli were small and lined by cuboidal epithelium in an abundant areolar stroma. In several areas, amorphous débris and pigment granules were visible in the bronchioles and air spaces. These could not be identified definitely as aspirated material.

In this case the third portion of the aortic arch was larger than the ductus arteriosus by a ratio of 6 to 5 (I/D = 1.2) in spite of the fact that the lungs were markedly hypoplastic.

COMMENT

The pulmonary arterial system of the fetal lung serves no apparent purpose other than supplying a portion of the nourishment of the lung. Therefore, provision is made for the major part of the circulating blood to by-pass this part of the vascular tree, shunts being provided by the foramen ovale and the ductus arteriosus.

Noback and Rehman[20] made a detailed dissection of the ductus arteriosus in fetal and newborn cadavers and stated that "The size of the ductus is much larger than is generally assumed, being equal to or greater in caliber than the aortic arch, the pulmonary artery, or the descending aorta." It is difficult to conceive of a branch (the ductus arteriosus) being larger than the trunk from which it originates (the pulmonary artery). In 26 fetal and newborn cadavers without gross abnormalities of the cardiovascular or respiratory systems, we obtained the measure-

ments shown in Table II, which do not agree with some of the findings of Noback and Rehman. Comparison of columns 3 and 4 shows that in only 6 of the 26 cases did the diameter of the ductus arteriosus exceed the diameter of the isthmus of the aortic arch, and in these cases the I/D ratio was 0.75 or greater.

In 11 instances the diameter of the aortic isthmus exceeded that of the ductus, and in the remaining 9 cases the two vessels were of equal size. The mean ductus size for the entire series was 5.8 mm. The mean isthmus size was 6.1 mm. The mean isthmus/ductus ratio for the series was 1.07 ± 0.27 (Text-Fig. C).

The I/D ratio in case 1 was 0.62, a deviation of 0.45 from the mean. The I/D ratio in case 2 was 0.50 or less, a deviation of 0.57 or more from the mean. While these differences are not great enough to be conclusive in so small a series, they are significantly suggestive. Anomalies preventing only pulmonary expansion are so rare as to preclude the accumulation of a large series in any one institution. Therefore we feel justified in publishing this report as a stimulus to other observers.

Barclay, Franklin, and Prichard[21] investigated the fetal and neonatal blood flow by means of kineradiographs of opaque media injected into the inferior and superior caval systems of fetal lambs delivered by cesarean section, injections being made both before and after interruption of the placental circulation and establishment of extra-uterine respiration. They demonstrated that all blood returning to the heart by way of the superior vena cava passes through the right side of the heart and out through the pulmonary artery to be distributed to the lungs and to the descending aorta through the ductus arteriosus. The stream of blood returning through the inferior vena cava is split by the anterior crescent of the foramen ovale, the greater part of the stream being diverted into the left atrium, and a lesser part continuing through the right side of the heart to be mixed with the blood from the superior vena cava. The blood entering the left heart from the foramen ovale and pulmonary veins is distributed to the coronary arteries, the head, and upper extremities by way of the branches from the aortic arch. What remains then joins the stream from the right heart at the junction of the ductus arteriosus with the aorta.

These studies indicate the paths of flow but do not give an accurate index of the relative amount of blood that passes through the right and left sides of the heart, a feature that is difficult to determine directly. Since the right ventricular muscle is approximately as large as the left at birth, we may infer that the intra-uterine work of the right heart has been equal to that of the left. On the other hand, in extra-uterine life

TABLE II

Actual and Proportionate Diameters of the Aortic Isthmus and Ductus Arteriosus in Fetuses and Newborn Infants

Case	Crown-heel length	Isthmus	Ductus	I/D ratio*	Cause of death	Anatomical diagnosis
	cm.	mm.	mm.			
1	16	2	2	1.00	Unknown	Macerated fetus; stillborn
2	21	3.5	2	1.75	Unknown	Stillborn
3	24	3	2	1.50	Intra-uterine sepsis	Septic placenta; interstitial pancreatitis; interstitial pneumonia; stillborn
4	27	4	3	1.33	Unknown	Macerated fetus; stillborn
5	28	4	3	1.33	Previability	Petechial hemorrhage about base of heart; prematurity
6	28	4	3	1.33	Meconium peritonitis	Meconium peritonitis; minimal interstitial pneumonia; stillborn
7	29	3	4	0.75	Unknown	Macerated fetus; stillborn
8	29.5	5	3	1.67	Previability	Prematurity; subepicardial petechiae; adrenal petechiae
9	30	4	3	1.33	Previability	Prematurity
10	33	8	8	1.00	Previability	Prematurity
11	40	6	4	1.50	Unknown	Petechial hemorrhages beneath visceral pleura; stillborn
12	42.5	7	5	1.40	Unknown	Minimal subtentorial hemorrhage; petechial hemorrhage, heart; pulmonary congestion
13	45	8	9	0.89	Intra-uterine sepsis	Petechial hemorrhage, heart and great vessels; pulmonary congestion; interstitial nephritis; acute myocarditis; stillborn
14	47	7	7	1.00	Unknown	Petechial hemorrhages of pleural cavities and heart, great vessels, and kidneys; stillborn
15	47	6	6	1.00	Intra-uterine asphyxia (premature separation of placenta)	Early acute glomerulonephritis; passive congestion of liver and spleen; pulmonary congestion; stillborn
16	47	7	7	1.00	Unknown	Petechial hemorrhage of pleura and pericardium; minimal intracranial hemorrhage; stillborn
17	49	9	10	0.90	Absence of kidneys and ureters	Absence of kidneys and ureters; pulmonary atelectasis and petechial hemorrhages; fibrosis of pancreas; interstitial myocarditis
18	49	5	5	1.00	Bilateral adrenal hemorrhage	Bilateral adrenal hemorrhage; petechial hemorrhage in heart, lungs, and kidneys; stillborn
19	49	8	6	1.33	Intracranial hemorrhage	Intracranial hemorrhage; cleft palate and harelip; subpericardial and subpleural petechial hemorrhage; hemorrhage into thymus; stillborn
20	49	8	8	1.00	Unknown	Petechial hemorrhages about the heart and great vessels; stillborn
21	51	8	9	0.89	Intracranial hemorrhage	Intracranial hemorrhage
22	51.5	8	8	1.00	Unknown	Petechial hemorrhage beneath serosa of heart, lungs, and about the thymus; stillborn
23	52	8	7	1.14	Meconium peritonitis	Meconium peritonitis; early bronchopneumonia; macerated fetus; stillborn
24	52	7	9	0.78	Intracranial hemorrhage	Intracranial hemorrhage; petechial hemorrhage, pericardium; polydactylism; Meckel's diverticulum; bronchopneumonia; stillborn
25	52	8	9	0.89	Unknown	Subpleural and subpericardial petechial hemorrhage; macerated fetus; stillborn
26	53	8	8	1.00	Neonatal sepsis	Bronchopneumonia; interstitial nephritis; interstitial myocarditis

* Diameter of the isthmus: diameter of the ductus arteriosus.

the muscle mass of the left ventricle rapidly exceeds that of the right ventricle, even though they pump equal volumes of blood, so we may assume that the postnatal work demand upon the right heart is correspondingly less than that placed on the left.

The usual explanations of fetal circulation are predicated upon a functionless fetal lung. The small demand for blood of a functionless lung should require only a fraction of the output of the fetal right heart, and therefore we would assume that both ventricles contribute about equally to the systemic and placental circulation. Since a relatively large proportion of the blood from the left ventricle is supplied to the head and upper extremities, while the stream from the right ventricle supplies only the lungs before contributing blood to the descending aorta, we would expect that, if only sufficient blood passed through the pulmonary vascular tree to nourish the lung, relatively more of the blood in the descending aorta would come from the right heart than from the left.

Fetal blood vessels vary in size according to the average intravascular pressure and volume of blood flow as evidenced by the alterations in relative vessel size in certain congenital lesions of the heart. For instance, in aortic valve atresia there is hypoplasia of the aorta. Thus vessel size is an indicator of the average relative pressure and volume flow carried by the vessel. On this basis, if the lung were normally inactive in utero, we would expect the ductus arteriosus to be larger than the isthmus of the aorta, since the volume of blood flowing through the ductus would be greater. This is not the usual finding. Abel and Windle[22] studied the blood content of fetal lungs before the beginning of respiration and after 1 to 24 hours of air breathing by calculating the hemoglobin content from determinations of the iron content of the ashed lung. They found no appreciable difference in the amount of blood in the lung before and after air breathing. They concluded that there is no sudden increase in the volume of pulmonary circulation with air breathing. They stated that "It appears that a circulation is already present in the lungs during late prenatal life which is wholly capable of caring for oxygenation pending the assumption of respiration." Therefore, we must look for other anatomical and physiologic factors in the later stages of development to explain the increasing diversion of blood into the pulmonary circulation.

Since, in general, organ systems that must function at birth show evidence of trials of function in utero (urine in the bladder, bile in the gallbladder, swallowed amniotic contents in the stomach), and organs that cannot function in utero (the eyes, for instance) show slow devel-

opment of function post-natally, it is reasonable to expect that an organ system so important to the survival of the individual as the respiratory apparatus should undertake intra-uterine trials of function. The portions of the respiratory apparatus that would be expected to benefit by intra-uterine respiratory movements are the chest muscles, the pulmonary alveoli, and the pulmonary vascular system. The effect upon the muscles would be difficult to evaluate. Potter and Bohlender[10] have shown that alveolar development does not depend upon the expansion of the lung, and our findings agree with theirs. We are concerned, then, with pulmonary vascular development.

After birth the change in intrapulmonary pressure with the onset of regular, rhythmic respiration is assumed to play a major rôle in diverting the total output of the right heart into the pulmonary circulation. The studies of Barclay, Franklin, and Prichard[21] show that the first change to take place in blood flow after birth is cessation of flow through the foramen ovale. This occurs almost immediately with the beginning of extra-uterine respiration. Flow through the ductus arteriosus continues for a few minutes longer, and stops more gradually.

The closure of the foramen ovale is accomplished by an increased flow of blood from the pulmonary veins, which increases the pressure in the left atrium and forces the posterior leaf of the foramen ovale against the anterior crescent to occlude the opening. Since this occurs before the flow in the ductus ceases, the expansion of the lungs and the resultant changes in pressure must play the major rôle in diverting blood from the ductus arteriosus into the pulmonary system. If pressure against the walls of the ductus, as suggested by Noback and Rehman,[20] or active contraction of the ductus, as suggested by Patten[18] and others, were the primary factors, we would expect the flow in the ductus to stop first.

Since deep, rhythmic, extra-uterine respiration produces profound changes in the circulatory system, it is reasonable to expect that intra-uterine respiratory movements, even though less active, would also have an effect on the pulmonary circulation of the fetus. Acting over a much longer period of time, this should produce changes in the relative blood flow through, and size of, the great vessels. Absence of intra-uterine respiratory movements should negate this effect.

It is hardly conceivable that a vascular network capable of handling the entire circulating volume of blood immediately after birth could develop without stimulus. Abel and Windle[22] pointed out that "studies by Thoma in 1894 (Keibel and Mall, '12) and later by Mall ('06) and

Flint ('06) have shown that arteries and veins are formed from capillary nets and represent a functional adaptation of the net to the demands of the circulation. In other words it is developmentally unsound to assume the existence of well developed pulmonary vessels which contain a volume of blood well below their capacity. The size of these vessels depends entirely on the volume of blood coursing through them and is a resultant adaptation thereto."

Hamilton, Woodbury, and Woods[23] stated: "To conceive of a capacious but virtually unused vascular plexus developing in the growing lungs—ready at the instant of birth to receive a suddenly re-routed current of blood carried to the lungs for oxygenation"[24]—may well smack of teleology. It seems more logical, therefore, to expect the development of the pulmonary vascular system to take place in response to gradually increasing functional demand. This demand could consist, most logically, of intra-uterine respiration-like movements resulting in the expansion of the lungs.

With the stimulus of lung expansion we would expect an increasing diversion of blood into the pulmonary vascular system with less blood remaining in the ductus arteriosus. This would balance the withdrawal of blood through the branches of the aortic arch and equalize the volume flow through the aortic isthmus and the ductus arteriosus. This would explain the usual size ratio between these two vessels.

If this is the case, interference with expansion of the lungs during intra-uterine life should result in an underdeveloped pulmonary vascular tree, which in turn should be reflected in a relative increase in the size of the ductus arteriosus, since less blood would circulate in the lungs and more would pass through the ductus. In case 1, one lung was hypoplastic and the infant was presumably unable to expand the lungs in utero because the absent diaphragm made it impossible to reduce the pressure within the chest. In this case the diameter of the ductus arteriosus exceeded the diameter of the aortic isthmus by a ratio of 8 to 5 (I/D ratio, 0.62) (Text-Fig. B, see page 415). It might be argued that the hypoplasia of the lung was entirely responsible for the reduced pulmonary blood flow.

In case 2, the lungs were of normal size and the alveoli were normally developed. The only functional impairment was interference with expansion of the lungs, and in this case the aortic isthmus was less than half the diameter of the ductus arteriosus (I/D ratio, 0.5 or less) (Text-Fig. B, see page 415). This suggests a markedly diminished pulmonary vascular development. Since the only function rendered impossible as

a result of the absent trachea was expansion of the lungs, we infer that the diminished pulmonary vascular development was the result of the absence of lung expansion. The fact that the aortic isthmus was reduced in size absolutely, as well as relatively, in both cases, suggests that a considerable proportion of the output of the left heart is derived from the return flow through the pulmonary veins, and if this is diminished for any reason, the flow through the foramen ovale is insufficient to maintain a normal volume flow through the left heart.

In case 3, the normal size ratio between the ductus arteriosus and the aortic isthmus was maintained in spite of hypoplastic and supposedly functionless lungs. However, in this case the brain, with its relatively enormous demand for blood, was absent. Less blood was withdrawn from the aorta and thus a larger blood volume was permitted to flow through the aortic isthmus, balancing the increased flow through the ductus arteriosus.

Work now in progress on certain arteriosclerotic changes in the ductus arteriosus appearing in the later months of gestation seems to support our theory further and will be reported in a later paper.

Patten[18] stated that "If, through any deficiency in the development of the lungs themselves, or of their vessels, undue resistance to the free passage of blood exists, the pulmonary artery will continue to shunt blood over the ductus arteriosus to the aorta just as it did before birth. That this happens but rarely is perhaps the most remarkable fact about the entire series of circulatory changes which take place at birth; for there is no way this peripheral part of the pulmonary circulation can be tested out under functional conditions while the fetus remains in utero."

We believe that intra-uterine respiration-like movements resulting in expansion of the lungs and the aspiration of amniotic fluid not only constitute a test of the peripheral pulmonary circulation under functional conditions in utero, but are necessary for the development of the pulmonary vascular system, making possible an adequate post-natal pulmonary circulation.

Summary

We have presented a hypothesis of a "functioning" fetal lung as a necessary stimulus to the development of the pulmonary vascular system. This hypothesis is based upon analysis of facts already known and upon measurements of the great vessels in 26 cases of fetal and neonatal death without pulmonary or cardiac anomalies. It is supported by a case with hypoplasia of one lung and interference with respiration due

to absence of the left diaphragm. There was an altered size ratio between the aortic isthmus and the ductus arteriosus, I/D = 0.62. The hypothesis is further supported by a second case with grossly normal pulmonary development but with absence of the trachea, which made expansion of the lungs impossible. Again the I/D was altered, *i.e.*, 0.5.

A third case, an anencephalic monster with an anomaly of the respiratory system that would not prevent intra-uterine respiration-like movements, showed normal vascular development. The absence of respiratory movements reported[9] with this condition is assumed to have been counterbalanced by the decreased circulation to the head.

Thus supported, our hypothesis becomes a theory. The theory will require many observations to confirm it; but on the basis of this study we offer the following conclusions subject to further verification.

Conclusions

The normal isthmus/ductus diameter ratio is approximately 1.

The average volume flow through the pulmonary vascular system prenatally is approximately equal to the volume of blood supplied to the head and upper extremities.

This suggests an active stimulus to pulmonary blood flow, which could consist, most logically, of respiration-like movements resulting in lung expansion with its concomitant reduction in pressure in the pulmonary vascular system.

The vascular anomaly in the first 2 cases indicates an alteration in blood flow resulting from a decreased pulmonary circulation. Since it was found to be associated with a malformation that prevented only intra-uterine pulmonary expansion, we conclude that interference with intra-uterine pulmonary expansion can be the sole cause of this vascular anomaly. If interference with a function produces abnormal development, that function must be physiologic. Therefore, intra-uterine respiration-like movements are a physiologic function, because free expansion of the lungs during fetal life is necessary for normal development of the pulmonary vascular system.

REFERENCES

1. Snyder, F. F., and Rosenfeld, M. Direct observation of intrauterine respiratory movements of fetus and rôle of carbon dioxide and oxygen in their regulation. *Am. J. Physiol.*, 1937, **119**, 153–166.
2. Snyder, F. F., and Rosenfeld, M. Breathing of amniotic fluid as a normal function of fetal respiration. *Proc. Soc. Exper. Biol. & Med.*, 1937, **36**, 45–46.

3. Snyder, F. F., and Rosenfeld, M. Intrauterine respiratory movements of the human fetus. *J. A. M. A.*, 1937, **108**, 1946–1948.
4. Barcroft, J., and Barron, D. H. Genesis of respiratory movements in foetus of sheep. *J. Physiol.*, 1936, **88**, 56–61.
5. Ahlfeld, F. Die intrauterine Tätigkeit der Thorax- und Zwerchfellmuskulatur. Intrauterine Atmung. *Monatschr. f. Geburtsh. u. Gynäk.*, 1905, **21**, 143–163.
6. Windle, W. F., Dragstedt, C. A., Murray, D. E., and Greene, R. R. A note on the respiration-like movements of the human fetus. *Surg., Gynec. & Obst.*, 1938, **66**, 987–988.
7. Reifferscheid, W., and Schmiemann, R. Röntgenologische Untersuchungen über die sogenannte intrauterine Atmung des Foeten. *Ber. Phys. med. Ges. Würzburg*, 1939, **62**, 202.
8. Davis, M. E., and Potter, E. L. Intrauterine respiration of the human fetus. *J. A. M. A.*, 1946, **131**, 1194–1201.
9. Zettelman, H. J. Initial fetal atelectasis. *Am. J. Obst. & Gynec.*, 1946, **51**, 241–245.
10. Potter, E. L., and Bohlender, G. P. Intrauterine respiration in relation to development of the fetal lung, with report of two unusual anomalies of the respiratory system. *Am. J. Obst. & Gynec.*, 1941, **42**, 14–22.
11. Potter, E. L. Personal communication.
12. Bremer, J. L. The diaphragm and diaphragmatic hernia. *Arch. Path.*, 1943, **36**, 539–549.
13. Marek, J. J. Congenital deformity of trachea. *Ohio State M. J.*, 1940, **36**, 1308.
14. Fritz, E. Eine seltene Missbildung der oberen Luftwege. (Teilweiser Mangel der Luftröhre, Verschluss des Kehlkopfes und Osophagotrachealfistel.) *Virchows Arch. f. path. Anat.*, 1933, **289**, 264–269.
15. Walcher. Angeborener Mangel der Trachea. *Deutsche Ztschr. f. d. ges. gerichtl. Med.*, 1928, **12**, 292–296.
16. Payne, W. A. Congenital absence of the trachea, specimen (illustrated). *Brooklyn M. J.*, 1900, **14**, 568.
17. Bailey, F. R., and Miller, A. M. Textbook of Embryology. William Wood & Co., New York, 1911, ed. 2, 362–370.
18. Patten, B. M. Human Embryology. The Blakiston Co., Philadelphia, 1947, pp. 682–697.
19. Gruenwald, P. A case of atresia of the esophagus combined with tracheo-esophageal fistula in a 9-mm. human embryo, and its embryological explanation. *Anat. Rec.*, 1940, **78**, 293–302.
20. Noback, G. J., and Rehman, I. The ductus arteriosus in the human fetus and newborn infant. *Anat. Rec.*, 1941, **81**, 505–527.
21. Barclay, A. E., Franklin, K. J., and Prichard, M. M. L. The Foetal Circulation and Cardiovascular System, and the Changes That They Undergo at Birth. Blackwell Scientific Publications, Ltd., Oxford, 1944, 275 pp.
22. Abel, S., and Windle, W. F. Relation of the volume of pulmonary circulation to respiration at birth. *Anat. Rec.*, 1939, **75**, 451–464.
23. Hamilton, W. F., Woodbury, R. A., and Woods, E. B. The relation between systemic and pulmonary blood pressures in the fetus. *Am. J. Physiol.*, 1937, **119**, 206–212.
24. Quoted in reference 23 from: Growth and development of child. Part II. Whitehouse Conference. Century Co., New York, 1933.

SARCOIDOSIS OF THE SPLEEN
Report of Four Cases with a Twenty-Three Year Follow-up in One Case *

Saul Kay, M.D.†
(From the Department of Surgery, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

This paper presents 4 cases of marked splenomegaly due to Boeck's sarcoid in which the diagnosis was not made until after splenectomy. In one case the diagnosis was suspected after an axillary lymph node had been removed and sectioned. It is intended to call attention to sarcoid as it exists predominantly in the spleen, making it mandatory to consider this disease in the differential diagnosis of splenomegaly. The histologic features of sarcoidosis of the spleen and its relation to Stengel-Wolbach's sclerosis will be discussed, as well as the peculiar intracellular "asteroid bodies" which were first described by Wolbach in 1911. The so-called "Schaumann bodies" were not seen in these cases. The latter were described by Schaumann in 1917, and consist of double-contoured, stratified, basophilic inclusions contained in the cytoplasm of giant cells or sometimes in intercellular spaces in relation to giant cells.

As far back as 1915, Kuznitzky and Bittorf regarded Boeck's sarcoid as a generalized disease. In 1919, Kettle, writing on splenomegaly, described a case (case 4) in which the lesions were like sarcoid. He further mentioned that such cases were similar to others described by Stengel (case 3) and Wilson. These latter authors both classified their cases as Gaucher's disease, although the lesions suggested tuberculosis.

Nickerson, in 1937, described the autopsy findings in 6 cases. The spleens were enlarged in 4, weighing 200, 230, 640, and 890 gm., respectively. In the same year, a classic paper by Longcope and Pierson on sarcoidosis appeared. Detailed descriptions of the lesions were given. "Asteroid bodies," however, were not mentioned.

Froehlich and Scherer reported the case of a 50-year-old woman whose spleen weighed 1027 gm. Systemic sarcoid disease was likewise present. Pautrier's case was that of a 40-year-old woman in whom the spleen was 26 cm. long and weighed 1700 gm. There were, however, also lesions in the skin, lymph nodes, and lungs. The hepatosplenic form of sarcoidosis was reported in a 1-year-old child by Glanzmann. A case of spontaneous rupture of the spleen in sarcoidosis was submitted by

* Received for publication, June 24, 1949.
† Trainee of The National Cancer Institute.

James and Wilson. Culligan and Snoddy reported a case of sarcoidosis of the spleen in which the weight was 2.5 lbs.

Friedman has pointed out that involvement of the spleen in the course of generalized sarcoidosis is fairly common, constituting the second most common site. He further added that a pure form in which only the spleen is involved is probably non-existent. However, when the spleen is enlarged and other findings are insignificant, a diagnosis of Banti's disease or tuberculosis is usually made.

In a more recent paper by Rosenthal and Feigin, post-mortem findings in 4 cases were presented. These authors emphasized the healing by fibrosis and hyalinization of the Boeck tubercle. An important comment was the fact that special stains showed the presence of reticulum fibers between the reticulum cells of the tubercle. Incidentally it was mentioned that the spleen need not be involved. "Asteroid bodies" were not described.

Latterly there has appeared a discussion by Teilum of the nature of the "Schaumann bodies" in sarcoidosis. This author considered these bodies and the "asteroid bodies" described by Friedman as essentially of the same nature. Teilum interpreted sarcoidosis as an allergic hyperglobulinosis with a globulin precipitate in the reticulo-endothelial system. The giant cell reaction is by consequence a reaction to the precipitation of a biochemical substance within reticulum cells.

The "Schaumann bodies" were seen recently by Scotti and McKeown in a case of sarcoidosis involving the heart, and Dutra has described these bodies in granulomas occurring in the lung in beryllium workers.

Report of Cases

Case 1 *

L. J. B. was a colored female, 27 years of age, who had entered the Presbyterian Hospital in the Medical Service in March, 1925, complaining of increasing weakness, a weight loss of 55 lbs. in 6 months, and anorexia. The symptoms had been present for 1 year. She also had dull aching pain in the left upper quadrant with progressive enlargement of the abdomen for the past 6 months. During this time her menses had appeared at intervals of 2 months.

On examination, the temperature was 100° F.; pulse, 90; respirations, 22. The mucous membranes were pale. The abdomen was soft but presented a large, smooth, flat, tender, hard mass in the left costal margin, extending to 4 cm. below the umbilicus. Liver dullness was 5 cm. below the costal margin, but the edge was not palpable.

Laboratory findings included a red blood cell count of 4.03 millions; hemoglobin, 75 per cent; white blood cells, 9,200, with normal differential count. Wassermann test of the blood was negative. Bleeding and clotting times were normal. Platelet count was 250,000. Mantoux test for tuberculosis was negative.

The clinical diagnosis was splenic anemia (Banti's) and a laparotomy was performed on the twelfth hospital day by Dr. A. O. Whipple. "The interesting findings

* Permission to use this case was granted by Dr. A. O. Whipple.

proved to be not in the spleen but in the liver. The liver was found remarkably mottled in a mosaic fashion with brownish, irregular areas on the background of normal liver colored tissue. The liver did not feel especially hard, however, or cirrhotic. The spleen was considerably enlarged, measuring over 20 cm. in its greatest diameter." The lower angle showed a few very small nodules. A splenectomy was done, and a specimen from the left lobe of the liver was obtained. The postoperative diagnosis was an unrecognized form of hepatitis.

Following complications of stitch abscess and bronchopneumonia the patient was discharged on the 29th postoperative day. Eleven months later she felt fine but tired easily. The patient has been followed until August 27, 1948, 23 years and 5 months after splenectomy. She has had various dermal, rectal, endocrine, urinary, dental, and dietary complaints, but they have not appeared to be serious. Physical examination showed no enlargement of lymph nodes. Roentgenograms of the chest were negative. Since 1929 she has become rather severely hypertensive (blood pressure, 240/130 mm. of Hg). At present she is being studied in the Group Clinic for osteo-arthritis and a possible colonic lesion. The serum globulin has been persistently elevated since 1941 (average, 3.6 gm. per 100 cc.). The patient, however, has not been seen for the last 6 months at the time of this writing.

Pathologic Findings

The specimen (A-31998) consisted of a spleen measuring 22.5 by 12 by 8 cm. and weighing 1250 gm. The capsule was bluish red and slightly nodular. A few fibrous adhesions were present. At various places on the capsule, small, gray, translucent areas, quite discrete and sharply circumscribed, were seen. These measured 1 to 2 mm. in diameter. The irregular contour of the capsule produced an appearance not unlike cirrhosis. On cut section, the splenic pulp was reddish blue and bulging. Small, irregularly shaped, gray, translucent areas surrounded by irregular zones of congestion were scattered throughout the pulp. A small piece of liver tissue, 7 by 7 by 3 mm., was submitted also.

Microscopic examination of the spleen showed that the pulp was everywhere filled with, and almost completely replaced by, tubercle-like collections featured by multinucleated giant cells containing 3 to 30 nuclei (Fig. 1). The groups contained from 2 to 6 giant cells on the average and were surrounded by a capsule of connective tissue which sent prolongations between the giant cells. Nowhere was there evidence of necrosis. Many of the giant cells contained within the cytoplasm peculiar star-shaped bodies (Fig. 2). Immediately about each star-like body, separating it from the cell cytoplasm, was a clear zone which could not be stained with any of the dyes used. In addition to these foreign bodies there were numerous rounded or ovoid spaces in the cell cytoplasm, about 5 to 7 μ in diameter. Sections from the liver showed the same giant cells in groups with similar cytoplasmic inclusions. Attempts to find bacteria with the Gram and Ziehl-Neelsen stains were unsuccessful, nor could any organisms be found by the Levaditi method. "Schaumann

bodies" were not identified. Examination of the sections with polarized light failed to reveal the presence of doubly refractile material.

Diagnosis. Boeck's sarcoid of spleen and liver.

Case 2 *

I. T. was a white female, 30 years old, who entered the Vanderbilt Clinic on July 17, 1944, complaining of loss of weight and pains in the left upper quadrant of 8 months' duration not related to meals. She had had morning vomiting spells, but no tarry stools. A gradual increase in weakness had been noted.

On examination, the patient was found to be chronically ill, showing weight loss and anemia. Epitrochlear and axillary nodes were palpable. The liver and spleen were enlarged. Pingueculae were noted in the sclerae. Temperature was 100.4° F.; pulse, 88; respirations, 20.

Laboratory tests disclosed a moderate anemia with leukopenia, with a total white blood cell count sometimes as low as 2300. The differential count was normal. Platelet count and fragility test were normal. Bone marrow obtained for biopsy showed no abnormal findings. The Klein test was negative. The plasma proteins were elevated with the globulin fraction elevated to 3.9 per cent. The cephalin flocculation was 3 plus but later became negative. Roentgenograms of the chest showed bilateral hilar lymph node enlargement and those of all bones showed no evidence of disease.

The clinical diagnosis was Banti's disease, malignant lymphoma, or Gaucher's disease. One month after admission an axillary node was removed and the pathologic diagnosis of Boeck's sarcoid was made. Nine days later the spleen, accessory spleen, and a piece of liver tissue were removed. A guinea-pig inoculated with splenic extract showed no evidence of tuberculosis when it was killed 1 month later.

After discharge from the hospital, the patient was followed in the Clinic. Her last visit was on October 7, 1947, 3 years and 2 months following splenectomy. Her blood counts had become normal, although she was still complaining of weakness. A mass present in the right upper quadrant was thought to be liver. The cephalin flocculation was 2 plus.

Pathologic Findings

The spleen (A-90612) was huge, weighing 1150 gm. and measuring 30 by 17 by 6 cm. (Fig. 3). The whole surface presented a series of mounds 3 to 4 mm. in diameter. The organ was firm. On sectioning, the cut surface was very coarsely pebbled, grayish, firm, and dry (Fig. 4). The accessory spleen measured about 2 cm. in greatest diameter, and the cut surface was finely mottled with white dots. The piece of liver measured 0.8 by 0.3 by 0.2 cm.

Sections of the spleen and accessory spleen showed the same changes microscopically. The disease was similar to that in the axillary node. There were many tubercle-like lesions, often with central multinucleated giant cells. There was no caseation necrosis, but a considerable degree of fibrosis was noted (Fig. 5). Many of the giant cells were huge, and contained large numbers of nuclei. They were sometimes centrally, sometimes peripherally, distributed. In a number of cells there were

* Permission to use this case was granted by Dr. R. H. E. Elliott.

cytoplasmic inclusions, typical star-like bodies (Fig. 6). "Schaumann bodies" were not identified. The liver tissue showed numerous non-caseating tubercles. These resembled those seen in the spleen. Doubly refractile material was not seen.

Diagnosis. Boeck's sarcoid of the spleen, accessory spleen, and liver.

Case 3 *

A. O. was a white female, 41 years old, a registered nurse, who was admitted to the New York Postgraduate Medical School and Hospital on August 1, 1944, because of vomiting of 2 weeks' duration.

The family history was non-contributory.

The patient had had "jaundice" once in childhood. Her two children were living and well. She had had the "flu" 4 or 5 months prior to admission, and had suffered vague malaise and weakness since that time.

With the onset of the present illness the patient began to vomit practically all food taken, without accompanying pain but with a heavy feeling in the left upper quadrant. There was chronic constipation. There was no history of hematemesis except for a few blood streaks on the day before admission. In the past 2 weeks the patient had lost 10 lbs.

On examination, the temperature was 99° F.; pulse, 88; respirations, 20; blood pressure, 122/80 mm. of Hg. There was a smooth, firm, movable mass in the left upper abdominal quadrant, with a 2 cm. area of tenderness near the midline. The mass was approximately 5.0 by 9.0 cm. and was presumed to be spleen.

On admission the red blood cell count was 3.93 millions with a hemoglobin of 62 per cent. The white blood cell count was 5,300 with a normal differential. The red cell fragility was slightly decreased. Platelets were 180,000. Chemical examination of the blood showed a reduction of chlorides. The CO_2-combining power was 65.2 vols. per cent. The icteric index was 4.0 units. The total cholesterol and cholesterol esters were 215 and 115 mg. per cent, respectively. The total blood proteins were 6.1 per cent with an albumin and globulin of 4.2 and 1.9 per cent, respectively. The alkaline phosphatase was normal. The urine was negative. The cephalin flocculation was 2 plus. Following injection of bromsulfalein there was 35 per cent retention after 30 minutes, and 5 per cent retention at the end of 1 hour. Roentgenograms of the chest showed a diffuse, reticular, soft and faint, miliary infiltration of both lungs. Roentgenologic examination of the bones was not made. The Mantoux test was negative.

The clinical diagnosis was splenomegaly of undetermined origin. A splenectomy was performed on the 15th hospital day. The postoperative course was uneventful, and the patient was discharged in a satisfactory condition on the 35th hospital day. She was next admitted to Mount Sinai Hospital of New York in September, 1948, and subsequently was lost to follow-up investigation.

Pathologic Findings

The gross specimen (no. 28639) consisted of an enlarged spleen measuring 18.5 by 10.2 by 5.5 cm. and weighing 710 gm. (Fig. 7). The surfaces were diffusely studded by moderately elevated, papular nodulations which varied from 1 to 22 mm. in diameter, but averaged between

* Permission to publish this case was granted by Dr. M. N. Richter. A discussion of this case, with different photographs, appears in Anderson's *Pathology*. A diagnosis of chronic granulomatous inflammation of undetermined etiology was made, and the publisher has kindly consented to the publication of this case as one of sarcoidosis of the spleen.

10 and 20 mm. These nodulations showed a certain tendency toward aggregations into groups. Most were not elevated over 5 mm. and were covered by an intact capsule. They were firm and of a tannish hue, the intervening surface being depressed, smooth, with a thin, translucent capsule through which the parenchyma appeared to be dark purple. No enlarged lymph nodes were found in the hilum. On cut section the entire parenchyma of the spleen was studded with these nodules, sometimes discrete, and sometimes forming aggregates suggesting fusion of individual nodules and measuring up to 3.5 cm. in diameter (Fig. 7). Most of the nodules were spherical and protruded above the cut surface. Their substance was firm, finely granular, and opaque, and was discolored various shades of light reddish brown or tannish gray. Sometimes in their central portion there were depressed, white, seemingly scarred areas, up to 8 mm. in diameter. The intervening splenic parenchyma was moderately firm but oozed blood and was homogeneously dark reddish purple.

Microscopic examination of the spleen showed that the nodules described grossly were composed of conglomerations of tubercles, varying in size and separated by variable amounts of splenic tissue corresponding to the intervening parenchyma seen grossly (Fig. 8). These tubercles consisted of groups of epithelioid cells associated with one or several multinucleated giant cells (Fig. 8). The latter were not typically of the Langhans' type, showing centrally grouped nuclei varying from 20 to 100 in number. Some of these giant cells contained vacuolated material within the cytoplasm, and occasionally a "star fish" inclusion was noted within a large vacuole. "Schaumann bodies" were not identified. Occasional small discrete tubercles and groups of epithelioid cells were scattered throughout the spleen; these generally were in association with malpighian corpuscles. The uninvolved splenic parenchyma showed dilated sinuses, relatively empty but sometimes containing small numbers of polymorphonuclear leukocytes which were seen also in the surrounding pulp. Many of the confluent tubercles showed central areas of fibrosis and fibrinoid degeneration. The tubercles also were circumscribed by concentric lamellae of fibrous scar tissue. No doubly refractile material was seen in any of the sections.

Diagnosis. Boeck's sarcoid of the spleen.

Case 4 *

W. W. was a Negro, 33 years old, who had been admitted to the Presbyterian Hospital on July 16, 1948, for splenomegaly of 2 years' duration.

Three years prior to admission bilateral glaucoma had developed, and he was

* Permission to use this case was granted by Dr. R. A. Deterling, Jr.

almost completely blind in both eyes. In 1933 he had developed chills and fever for which quinine was administered. Malarial parasites, however, were not found. The spleen was first palpable 2 years before, and grew steadily larger until it had reached the iliac crest. One year before the patient started having elevations of temperature up to 102° F., present only in the evening and never on consecutive days. In 2 years he had lost 30 lbs. of weight. Radiotherapy was administered to the splenic area about 1 year ago, but the exact dosage could not be ascertained.

The patient entered because of splenic enlargement, abdominal pains, and elevated temperature. He was an asthenic, poorly nourished Negro having abdominal pains. Shotty inguinal and axillary nodes were palpable. The spleen was palpable down to 5 cm. above the pubis. There was pain and tenderness over the splenic area. The temperature on admission was 100.6° F.

Several blood counts showed red cells in the vicinity of 3 million with the hemoglobin averaging about 10 gm. The white blood cells were reduced to 2000 to 3000 with a normal differential count. The reticulocyte count was 6.1 per cent; platelets, 140,000. No sickling was seen in 24 hours. The fragility test was normal. Bone marrow studies showed hyperactive erythropoiesis with a left-shift granulopoiesis. The sedimentation rate was 10 mm. per hour. The Kline test was negative. A skin test for echinococcus was negative. The Mantoux test (1:1000) was negative. The stool gave a negative guaiac test and there were no ova or parasites. Liver function tests were normal. The plasma globulin was not elevated. A chest film showed prominent hilar markings. A flat plate of the abdomen showed two calcified areas in the region of the right kidney, possibly renal calculi, and small areas of calcification in the left abdomen, possibly lymph nodes. Roentgenograms of all bones were negative except for several areas of diminished density in the radial head. No esophageal varices were seen on fluoroscopy.

The preoperative diagnosis was splenomegaly of undetermined origin. Splenectomy was done on the 17th hospital day and several accessory spleens were removed also. One of these was located at the bifurcation of the aorta. Following the operation the temperature became normal and remained so until the day of discharge. On the 34th hospital day the patient developed herpetic lesions on the penis, which were biopsied but found to be negative for sarcoid. He was discharged on the 40th hospital day in good condition. The patient was last heard from by mail on December 29, 1948, at which time he was in apparently good health.

Pathologic Findings

The gross specimen (A-8194) consisted of a huge spleen weighing 4800 gm. and measuring 35 by 15 by 12 cm. The splenic capsule was smooth and glistening, and the consistency of the spleen firm. On cut section there was a homogeneous grayish red surface which was rather dry and did not scrape easily. Lymphoid follicles and trabeculae were not distinguished. In the superior pole a sharply demarcated yellow infarct, 1 cm. in diameter, was present beneath the capsule. After formalin fixation of part of the specimen, and with the aid of a hand lens, it was noted that the cut surface was studded with minute, white, irregular areas with red centers. In addition to the main specimen there were several accessory spleens, the largest of which was 2.5 cm. in diameter and stated to be from the region of the bifurcation of the aorta. A small piece of the liver was likewise submitted for biopsy.

Portions from the spleen and liver were fixed in Zenker's solution and stained with hematoxylin and eosin, as well as with the trichrome and Laidlaw's methods for reticulin. The spleen and accessory spleens showed similar features. A large part of the parenchyma was replaced by discrete tubercle-like lesions which varied in size and were composed of epithelioid cells arranged in palisade fashion, often associated with multinucleated giant cells. These tubercles showed no caseation necrosis but sometimes showed central hemorrhage and fibrinoid degeneration. They often surrounded small central capillaries which were in turn surrounded by a small amount of collagen (Fig. 9). The giant cells contained few or numerous nuclei which were generally diffusely arranged throughout the cytoplasm. Within the cytoplasm, vacuolated material, red blood cells, and fibrinoid material often were found. The last was occasionally arranged in small focal clumps with a superficial resemblance to developing "asteroid bodies" (Fig. 10). Fully developed bodies, however, were not seen with careful search; nor were any "Schaumann bodies" noted. Surrounding the tubercles there were scattered areas of hemorrhage, fibrosis, and small diffuse infiltrations of lymphocytes associated with a few eosinophils. The Laidlaw stain showed abundant reticulin fibers circumscribing the tubercles. Fibers, however, also were seen between epithelioid cells, and sometimes appeared to radiate from a central blood vessel. The splenic tissue uninvolved by tubercles showed dilated sinuses lined by prominent littoral cells. The sinuses were generally empty but sometimes contained a few lymphocytes. A Ziehl-Neelsen stain of smears from the spleen was negative for acid-fast bacilli. The small piece of liver likewise showed a few tubercles scattered throughout the parenchyma. These were generally in portal and central areas, but were not associated with giant cells. The use of polarized light revealed no doubly refractile material.

Diagnosis. Boeck's sarcoid of spleen, accessory spleens, and liver.

Discussion

Clinically, these 4 cases have certain features in common. They all presented marked splenomegaly as the predominant physical finding, which was associated with weight loss, weakness, and various gastrointestinal complaints. As far as could be determined, lesions were also predominantly limited to the spleen, although cases 1 and 4 showed hepatic involvement also, and case 2 showed changes in the liver and lymph nodes. No bone lesions could be seen on x-ray examination, and only case 3 showed suggestive pulmonary sarcoid lesions.

From a pathologic point of view, the spleens of all cases were similar,

with only minor differences in case 3. Grossly, case 3 showed coarse nodules reminiscent of nodular cirrhosis of the liver. These nodules were seen in microscopic sections to be due to confluence of the tubercular lesions with large portions of uninvolved spleen remaining. The other cases showed practically complete replacement of splenic tissue by fairly discrete tubercles, each representing circumscribed entities.

Fully developed "asteroid bodies" were seen only in cases 1 and 2. Case 4 showed giant cell inclusions not unlike developing asteroid bodies, and case 3 showed an occasional inclusion.

In 1938 Robb-Smith discussed various histologic changes in diseased lymph nodes and spleens. Under the heading "sinus reticuloses" he classified giant cell histiocytic sinus reticulosis or Stengel-Wolbach sclerosis. This lesion, he stated, was seen most commonly in the spleen, and closely resembled a diffuse chronic granulomatous tuberculosis. The point that is difficult to follow is Robb-Smith's attempt to distinguish Stengel-Wolbach's sclerosis from sarcoidosis. He maintains that in the latter, the reticulum fibrils do not run between the epithelioid cells which tend to merge into one another, in contrast to the discrete spindle-shaped histiocytes of the former lesion. A further point of difference was found in that in sarcoidosis there is a tendency to concentric cellular arrangement in the foci, which is never seen in Stengel-Wolbach's sclerosis.

I cannot agree with Robb-Smith concerning these fibers as a differential point. As can be seen from Figures 1 and 9, reticulum fibers often are found between individual epithelioid cells. While case 1 particularly showed concentric concentrations of reticulum around individual tubercles (Fig. 1), many tubercles also showed a variable number of fibers between individual cells. In fact, I believe the reason sarcoid tubercles do not caseate is the presence of central capillary blood vessels in the sarcoid tubercles (Fig. 9). These blood vessels maintain the blood supply of the tubercle and become surrounded by reticulum and collagen, which is minimal in early stages, and more abundant as the lesions develop. The tubercle of sarcoid in its healed stage is collagenized.

In tuberculosis, both a productive and exudative form is recognized. It is common knowledge that a tuberculous tubercle, particularly in the productive form, can produce its own reticulum. Consequently, it is difficult to see how differentiation based upon the presence or absence of reticulum can be maintained. It is my belief that whatever the nature of sarcoidosis, it and Stengel-Wolbach's sclerosis are one and the same.

The presence of "asteroid bodies" in multinucleated giant cells

has long been known. Wolbach described these inclusions in 1911, and their description has not since been improved upon. The nature of these bodies is still questionable, but most authors agree that they stain like fibrin in some instances. To Friedman's excellent account of the staining and chemical reactions of "asteroids," only a few pertinent facts can be added. With the use of the Levaditi stain in the first case it was possible to impregnate the "asteroid body" with silver. Gram and Ziehl-Neelsen stains both failed to stain these bodies on repeated occasions. The results with the Levaditi and Gram stains make it seem unlikely that the "asteroids" are chemically identical with fibrin.

A rather enticing view is that since central fibrinoid degeneration is often seen in sarcoid tubercles, the giant cells act as macrophages and engulf some of the fibrinoid material. This may explain the particles of material of various sizes and shapes often seen in the cytoplasm of giant cells, some of the particles ultimately leading to a "fully developed asteroid body" (Figs. 2, 6, and 10). The presence of vacuoles and red cells near the inclusions makes this idea particularly attractive.

Contrary to views held by some authors that "asteroid bodies" are seen only in sarcoid lesions, it may be stated that these bodies have been seen by Stout in conditions entirely unrelated to sarcoidosis. They have been found by chance in the following lesions: omental cyst; fibromyoma of uterus; giant cells of leprosy; giant cells containing paraffin particles; in the lungs and lymph nodes of 2 individuals dying of cancer of the colon and mandible, respectively; in the lungs and lymph nodes of a patient with pernicious anemia; in the spleen, liver, and lymph nodes of a thyroid case; in the lung, spleen, and liver of a patient with chronic nephritis, dying of apoplexy; and in the tonsil in a case of recurring tonsillitis. Even giant cells of tuberculosis of the Langhans' type may occasionally contain inclusions not unlike developing "asteroids" (Fig. 11).

A search was made for "asteroid" bodies in spleens removed in the Department of Surgical Pathology at Presbyterian Hospital, New York. This included 18 cases of undiagnosed conditions of the spleen and 36 examples of splenomegaly due to various causes. Approximately 200 sections were studied, and in none were these bodies found.

Summary

This study is based upon 4 cases of marked splenomegaly due to Boeck's sarcoid, in which the diagnosis was not made before pathologic examination.

Involvement of the spleen in generalized sarcoidosis is a fairly common event, and sarcoid lesions occurring almost exclusively in the spleen are frequent enough to merit consideration of sarcoidosis in the differential diagnosis of splenomegaly.

It is concluded that Stengel-Wolbach's sclerosis and sarcoidosis are the same entity.

"Asteroid bodies" or similar inclusions were found in giant cells in all 4 cases. They are thought to be fibrinoid inclusion bodies and of no special significance, being found in a number of other conditions unrelated to sarcoid.

I wish to express my thanks to Drs. A. P. Stout, M. N. Richter, and R. Lattes, whose many helpful suggestions have made this paper possible.

Addendum

Since the submission of this paper, additional information concerning case 3 (A. O.) has been made available through the courtesy of Mt. Sinai and St. Francis Hospitals of New York City. There were three admissions to the former hospital in 1948. By this time the patient had developed generalized sarcoidosis as evidenced by marked liver enlargement, generalized lymphadenopathy, and bilateral interstitial infiltrations throughout the lungs. At the first admission, the patient was treated moderately successfully with nitrogen mustard. A course of promine intravenously (total, 53 gm.) was given during the second admission without any marked improvement. During her third admission, a tender mass in the left axilla was excised which showed findings consistent with calcified bursitis. A lymph node, removed while the patient was in the hospital at one of her admissions, was examined by me and showed sarcoidosis. An interesting feature was the presence of doubly refractile foreign material associated with one of the multinucleated giant cells. Following discharge, the patient continued to have weakness, dyspnea, anorexia, and itching. She had lost considerable weight, and was admitted to St. Francis Hospital in August, 1949, complaining of a soft tissue swelling of the dorsal aspect of the right hand. Roentgenograms showed an inflammatory, destructive process of the terminal end of the fourth metacarpal bone. There was evidence of a pathologic fracture without repair. The patient has continued to remain weak, listless and without appetite. A cutaneous test for tuberculosis has remained negative.

Bibliography

Briggs, J. F. Systemic sarcoidosis. *Minnesota Med.*, 1945, **28**, 452–454.

Culligan, J. M., and Snoddy, W. T. Sarcoid of spleen. *Minnesota Med.*, 1945, **28**, 568–569.

Dutra, F. R. The pneumonitis and granulomatosis peculiar to beryllium workers. *Am. J. Path.*, 1948, **24**, 1137–1165.

Friedman, M. Sarcoidosis of the spleen. Report of a case with autopsy and a study of intracellular "asteroid bodies." *Am. J. Path.*, 1944, **20**, 621–635.

Froehlich, A., and Scherer, H. J. Zur Kenntnis der visceralen Form der Besnier-Boeckschen Krankheit. *Gastroenterologia*, 1940, **65**, 36–51.

Glanzmann, E. Die viscerale Form der Besnier-Boeck-Schaumannschen Krankheit beim Kind. *Ann. paediat.*, 1945, **165**, 125–141.

James, I., and Wilson, A. J. Spontaneous rupture of spleen in sarcoidosis. *Brit. J. Surg.*, 1946, **33**, 280–282.

Kettle, E. H. The splenic lesions in some cases of splenomegaly associated with secondary anaemia. *J. Path. & Bact.*, 1919–20, **23**, 413–424.

Kuznitzky, E., and Bittorf, A. Boecksches Sarkoid mit Beteiligung innerer Organe. *München. med. Wchnschr.*, 1915, **62**, 1349–1353.

Longcope, W. T., and Pierson, J. W. Boeck's sarcoid (sarcoidosis). *Bull. Johns Hopkins Hosp.*, 1937, **60**, 223–296.

Longcope, W. T. Sarcoidosis, or Besnier-Boeck-Schaumann disease. *J. A. M. A.*, 1941, **117**, 1321–1327.

Nickerson, D. A. Boeck's sarcoid. Report of six cases in which autopsies were made. *Arch. Path.*, 1937, **24**, 19–29.

Pautrier, L. M. La maladie de Besnier-Boeck-Schaumann. Masson & Cie., Paris, 1940, 344 pp.

Refvem, O. I. Chronic granulomas in the alimentary tract caused by minute mineral particles. II. "Boeck's disease" and occurrence of minute mineral particles. *Acta path. et microbiol. Scandinav.*, 1948, **25**, 107–121.

Richter, M. N. The Spleen, Lymph Nodes, and Reticulo-Endothelial System. In: Anderson, W. A. D. (ed.) Pathology. The C. V. Mosby Co., St. Louis, 1948, pp. 983–984.

Robb-Smith, A. H. T. Reticulosis and reticulosarcoma: a histological classification. *J. Path. & Bact.*, 1938, **47**, 457–480.

Rosenthal, J., and Feigin, I. Boeck's disease (Boeck's sarcoid). *Arch. Path.*, 1948, **45**, 681–693.

Schaumann, J. Recherches sur le lupus pernio et ses relations avec les sarcoïdes cutanées et sous-cutanées. *Nord. med. Ark.*, 1916–17, **49**, Pt. 2, No. 17, 81 pp.

Scotti, T. M., and McKeown, C. E. Sarcoidosis involving the heart. Report of case with sudden death. *Arch. Path.*, 1948, **46**, 289–300.

Stengel, A. Varieties of splenic anemia. *Tr. A. Am. Physicians*, 1904, **19**, 174–206.

Stout, A. P. Personal communication.

Teilum, G. The nature of the double-contoured and stratified intracellular bodies in sarcoidosis (Boeck-Schaumann). *Am. J. Path.*, 1949, **25**, 85–91.

Wilson, L. B. The pathology of splenomegaly: a study of the operative and autopsy material from the Mayo clinic. *Surg., Gynec. & Obst.*, 1913, **16**, 240–251.

Wolbach, S. B. A new type of cell inclusion, not parasitic, associated with disseminated granulomatous lesions. *J. M. Research*, 1911, **24**, 243–257.

DESCRIPTION OF PLATES

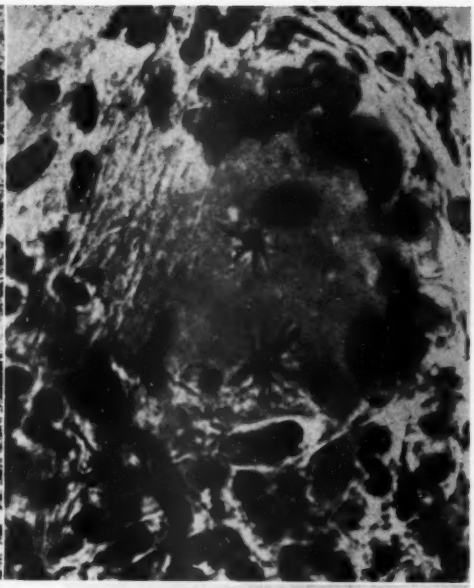
PLATE 64

FIG. 1. Case 1. Sarcoid nodules in the spleen. Levaditi stain showing reticulum framework.

FIG. 2. Intracellular "asteroids" in a giant cell of case 1. Hematoxylin and eosin stain.

FIG. 3. Case 2. Outer surface of the spleen.

FIG. 4. Cut surface of the spleen in case 2.



2

4

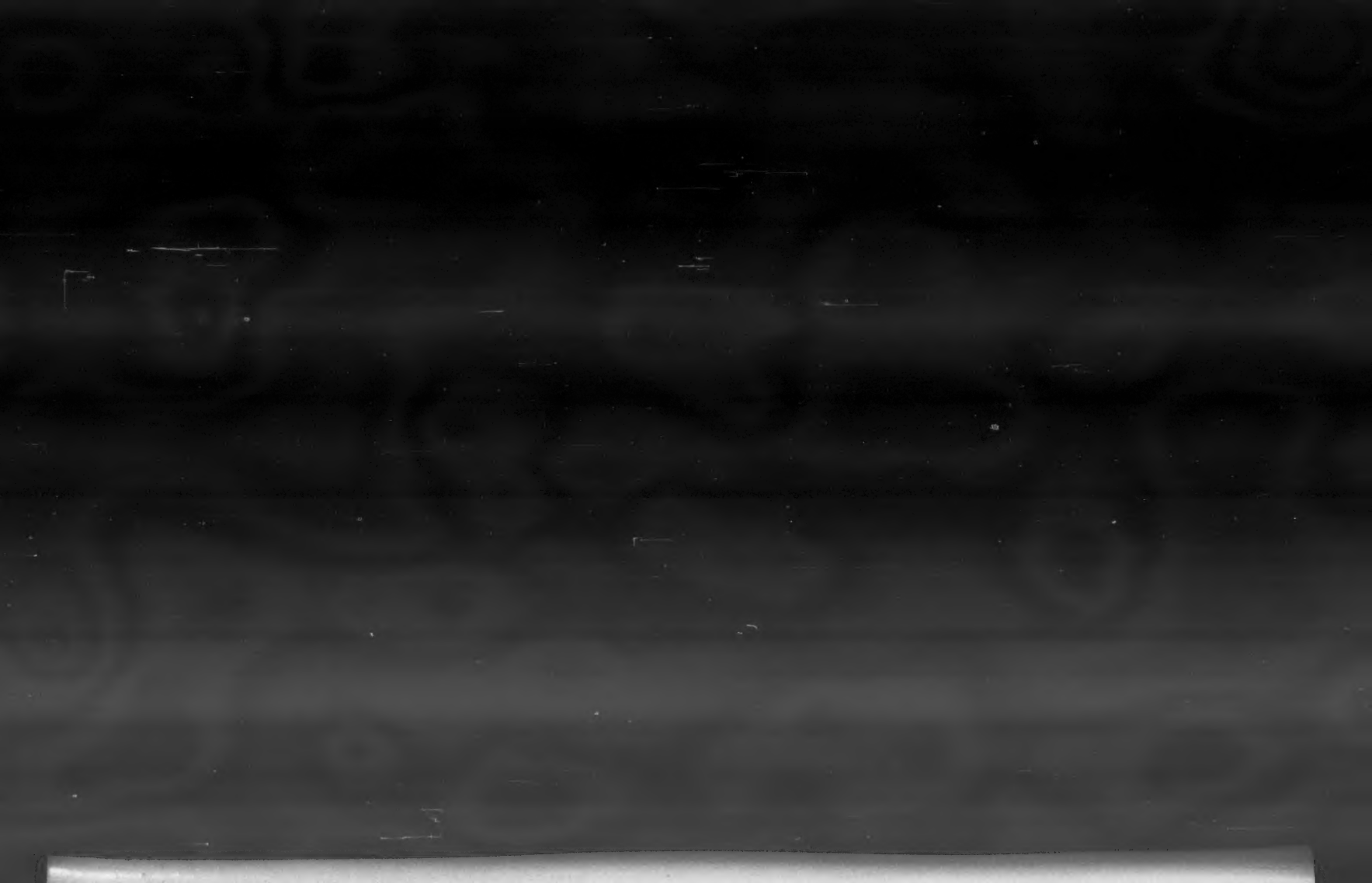
Kay Sarcoidosis of the Spleen

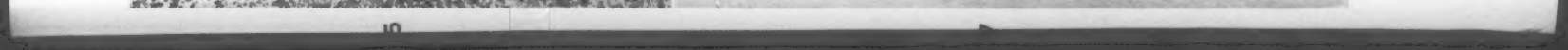
PLATE 65

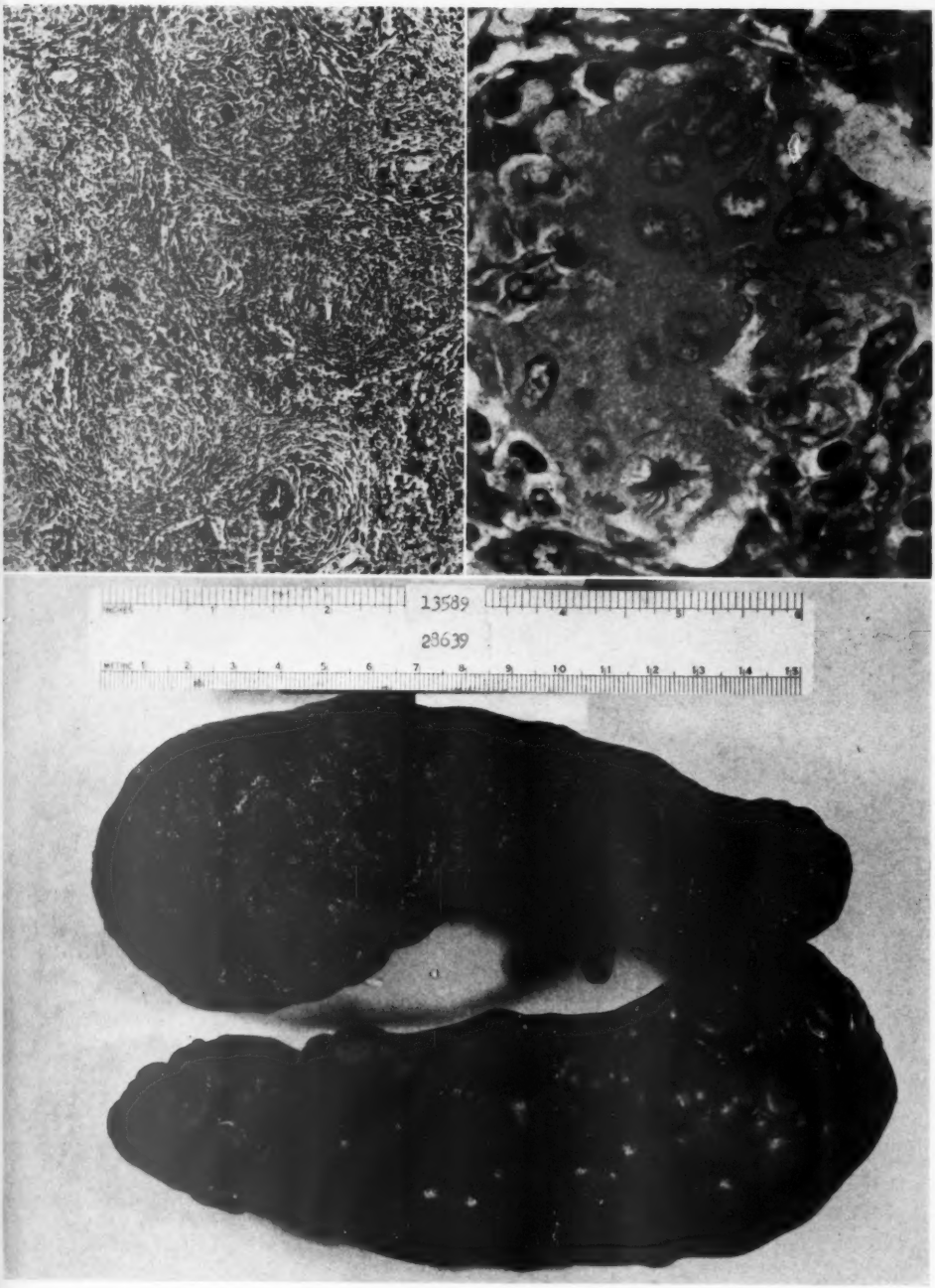
FIG. 5. Case 2. Sarcoid nodules in the spleen. Hematoxylin and eosin stain.

FIG. 6. Intracellular "asteroids" in a giant cell of case 2. Trichrome stain.

FIG. 7. Case 3. Outer and cut surface of the spleen.







Kay Sarcoidosis of the Spleen

PLATE 66

FIG. 8. Case 3. Sarcoid nodules partially replacing the spleen.

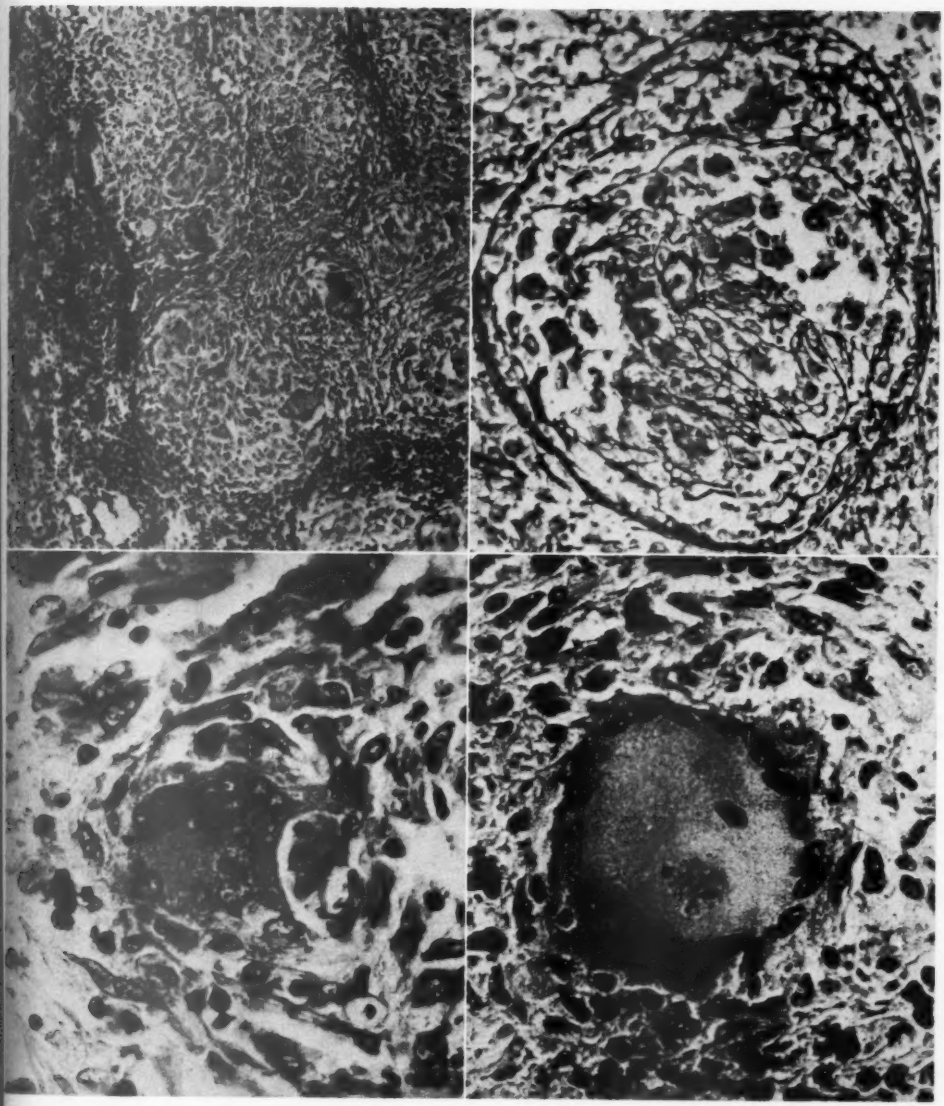
FIG. 9. Case 4. Reticulum framework of a sarcoid tubercle in the spleen, showing central blood vessel. Laidlaw's stain.

FIG. 10. Intracellular inclusion in a giant cell of case 4. Hematoxylin and eosin stain.

FIG. 11. Intracellular inclusion in a giant cell of Langhans' type in a known case of tuberculosis of a cervical lymph node. Hematoxylin and eosin stain.

8

9



10

11

Kay Sarcoidosis of the Spleen

PLASMA CELL TUMORS OF THE UPPER RESPIRATORY TRACT
A Clinico-pathologic Study with Emphasis on Criteria for Histologic Diagnosis *

Arnold J. Rawson, M.D., Paul W. Eyler, M.D.,† and Robert C. Horn, Jr., M.D.
(From the Laboratory of Surgical Pathology, the Department of Radiology, and the Penn Mutual Life Insurance Company Foundation for the Study of Neoplastic Disease, Hospital of the University of Pennsylvania, Philadelphia, Pa.)

Plasma cell tumors occurring either as discrete nodules in bone or diffusely spread through the bone marrow present a familiar histopathologic picture to the pathologist. Plasma cell tumors occurring in the soft tissues are encountered less frequently and offer the pathologist a considerably more difficult diagnostic problem. The type cell of such lesions frequently differs but little from the plasma cell that forms a conspicuous feature of many inflammatory reactions and the difficulty is further enhanced by the fact that plasma cells may predominate heavily in some inflammatory or granulomatous lesions, especially in the upper air and food passages. When the soft tissue lesions are associated with demonstrable bone involvement, diagnosis is not difficult. The problem becomes most acute when there is no associated bone involvement or when the latter cannot be demonstrated by clinical means. Hellwig,[1] in his extensive and careful review of the subject of extramedullary plasma cell tumors in 1943, concluded that one often cannot distinguish, on histologic grounds, between benign or inflammatory plasma cell tumors on the one hand and soft tissue manifestations of multiple myeloma or metastasizing plasma cell tumors on the other.

We have studied several plasma cell lesions involving the upper respiratory and food passages in which the clinical evolution of the disease has been followed. This study, which forms the basis of this report, has yielded information that is helpful in recognizing those extramedullary plasma cell tumors that may be expected to behave as malignant neoplasms.

Material

The material of the Laboratory of Surgical Pathology of the Hospital of the University of Pennsylvania includes 9 plasma cell tumors which, at least in their initial manifestations, were in the upper respiratory or alimentary tracts. Nine cases of primarily medullary plasma cell tumors (multiple myeloma), and numerous miscellaneous inflammatory lesions,

* Received for publication, April 25, 1949.
† Trainee of the National Cancer Institute.

from various parts of the body, which were composed largely of plasma cells, have been included in this study for purposes of comparison.

All of the tissues were fixed in Bouin's solution, or in 10 per cent formalin prior to sectioning, and for that reason special plasma cell stains were not successful. Sections stained by hematoxylin and eosin were examined for each of a number of details relating to pattern of the tumor, nuclear structure, and the presence of concomitant non-neoplastic features. Laidlaw stains were utilized to aid in the study of the stromal patterns.

Report of Cases

Case 1

H. G. was a white male, 43 years old, who had had a left radical mastoidectomy in 1931, followed by drainage for 14 years. Early in 1945 the discharge increased markedly and was associated with dizzy spells but no pain. The left ear was deaf. At operation a large amount of foul material and a polyp were removed from the left mastoid antrum. The wound healed and the patient has remained well to January, 1949. Sections of the polyp showed loose, edematous granulation tissue containing large numbers of plasma cells, many Russell bodies, and scattered inflammatory cells of other types. The plasma cells showed no definite orientation, but were haphazardly distributed throughout granulation tissue. The cytologic details of the plasma cells were like those seen in inflammatory lesions in general. A rare multinucleated cell was present, but no prominent nucleoli, mitotic figures, or giant nuclei were seen. The lesion was interpreted as a granuloma rather than a true neoplasm.

Case 2

F. F., a colored male, 45 years of age, was admitted to the surgical clinic of the Hospital of the University of Pennsylvania in October, 1947, with a history of weight loss of 25 lbs. over a period of several months. He had also had a mass involving the gum of the left mandible for 1 month. The teeth were carious and poorly aligned and in the lower left molar area was a firm, somewhat lobulated, non-tender, non-bleeding, pink, chestnut-sized mass, at the inner aspect of which were remnants of a tooth. No enlarged lymph nodes were palpated in the neck. The mass was removed surgically. Examination in January, 1949, revealed no evidence of local recurrence or of distant spread. The previous weight loss had been regained. Sections of the tumor showed a dense fibrous stroma containing small groups of typical plasma cells without any particular orientation. Numerous Russell bodies were noted but none of the cytologic characteristics of malignancy were seen. The lesion was interpreted as a granuloma.

Case 3

O. G., a white male, 33 years old, underwent a Caldwell-Luc procedure in March, 1946, for left nasal obstruction and discharge of 4 years' duration. Several polyps were removed from the left antrum. Following the operation the nasal discharge decreased but did not cease and was accompanied by a small amount of bloody oozing for several weeks. Because of unusually great vascularity, the margin of the Caldwell-Luc stoma was cauterized three times with trichloracetic acid. Radiation treatment was advised but refused. The patient experienced no further trouble and was well when last seen in January, 1949. The polyps had a histologic appearance similar to that of case 1 and, likewise, were regarded as inflammatory lesions.

Case 4

W. C. was a white male, 52 years old, who had first complained of nasal obstruction in May, 1945. This was relieved by removal of a polypoid mass from the nasal cavity. In April, 1946, he developed swelling at the base of the nose and obstruction of the left naris. A second polyp was partially extirpated and the patient was referred for radiation therapy. Examination in June, 1946, revealed a mass almost completely occluding the left nasal cavity. Roentgenograms of the sinuses showed a rounded lesion occupying the lower third of the left antrum which could not be shown to be connected to the intranasal mass. No bone invasion was demonstrated at that time. Roentgen therapy was directed to the left nasal cavity using 200 kv. filtered by 0.5 mm. Cu and 1.0 mm. Al. A total dose of 1950 r. (air) was administered in 8 days. During the next several months considerable regression of the intranasal tumor was observed. In January, 1948, radium was inserted through a Caldwell-Luc opening into residual tumor in the left antrum. Beginning in March, 1948, there became evident almost innumerable distant foci of tumor in the skeleton, subcutaneous tissues, and liver. Roentgen treatment was given to many of these. Usually a dose of about 1500 r. (air) was given to each, some being controlled quite well by this method. The patient has been ambulatory and able to continue with his work during most of this time, although new foci continue to appear. The serum proteins have ranged between 6.5 and 8.0 gm. per 100 cc. with a reversal of the albumin-globulin ratio. No albumin or Bence-Jones protein was ever found in the urine. In January, 1949, a tracer study was done with radioactive phosphorus (P^{32}) but there was insufficient uptake to warrant treatment with the isotope. A course of nitrogen mustard, methyl-*bis* (beta-chloroethyl) amine hydrochloride, was then given (17.6 mg. in 4 days) and a marked generalized improvement noted. He was last seen on February 8, 1949.

Tissues taken for biopsy from the nasal cavity, maxillary sinus, clavicle, and subcutaneous nodules all had a similar histologic appearance. Large masses of plasma cells were divided into groups by a delicate stroma consisting essentially of small capillaries. The plasma cells were massed in broad sheets with relation to this stroma. Their nuclei were relatively large with poor cartwheel formation, prominent nuclear membranes, and dispersion of the chromatin. No mitotic figures were noted. A rare, large, red-staining nucleolus was seen. Many giant nuclei and multinucleated cells were present.

The clinical course of this patient has provided firm support for the histopathologic interpretation of this lesion as a malignant plasma cell neoplasm.

Case 5

M. H., a white female, 83 years old, was first seen on March 6, 1946, with the complaint of right nasal obstruction of 4 weeks' duration. A soft gray mass was seen to fill the nasal cavity and appeared to arise from the middle turbinate. Roentgenograms revealed a small rounded shadow in the floor of the right antrum. No bone lesions were evident on roentgenologic examination and Bence-Jones proteinuria was not detected. Biopsy of the nasal mass showed broad sheets of plasma cells oriented on a delicate fibrous and vascular stroma. These invaded and replaced fibrous tissue. The nuclei were considerably larger than those of the usual plasma cell. Cartwheel formation was poor; nuclear membranes were prominent and there was some chromatin dispersion. Mitotic figures and multinucleated cells were not observed. Giant nuclei were rare. Moderately large, red-staining nucleoli were frequent. The lesion was considered to be a malignant plasmacytoma.

Treatment consisted of 200 kv. of roentgen irradiation through a single portal

over the right maxillary sinus and nasal cavity. In increments of 200 r. (air), 1250 r. (depth, 4 cm.) were given in 17 days. Within the following 2 months three implantations of two mc. radon seeds were made in the residual tumor in the right nasal cavity. By October, 1946, the nasal tumor had disappeared. In June, 1948, roentgen study of the right antrum again showed the rounded shadow previously observed, although there was no evidence of nasal tumor. The antral mass was thought to be a mucous cyst having no relationship to the nasal tumor. It did not respond to radiation as did the nasal lesion.

Case 6

L. S. was a white male, 73 years of age, who had been subjected to surgery for the removal of tissue obstructing the nasal passages in 1944. Shortly thereafter a partial cervical dissection was done because of enlarged lymph nodes. Roentgen treatment was then given to both sides of the neck. In 1945, the nasal obstruction recurred twice and each time more tissue was removed surgically from the nose. In December, 1947, when nasal obstruction again developed, he entered the Hospital of the University of Pennsylvania for the first time. Tissue was taken for biopsy and, after negative roentgen examinations of the skull and chest, treatment was begun with radium, using the Blady applicator. In 48 hours, 220 γ r. were delivered to the lesion in the nasopharynx. Three weeks later the mass was barely discernible. Histologically, the tumor had a suggestively papillary pattern, the neoplastic cells being in sheets on a delicate vascular stroma. The cells bore a strong resemblance to plasma cells, the nuclei showing fair cartwheel formation, and multinucleated cells were noted. The lesion was considered to be a malignant plasma cell neoplasm. Follow-up examination on January 25, 1949, showed no evidence of regrowth of the tumor.

Case 7

E. C., a white female, 59 years old, had a "cyst-like" growth removed from the left aryepiglottic fold and left ventricular band in 1938. At the same time there was noted a thickening along the left anterior faucial pillar. A specimen from the latter, taken the following year, had an appearance not unlike that of subsequent specimens. In 1943, the laryngeal lesion had recurred and was removed by laryngofissure. It recurred again in 1 month and was accompanied by enlargement of the left posterior cervical nodes. In June, 1946, the patient was admitted to the Hospital of the University of Pennsylvania complaining of a sore tongue and "stuffy nose." Her weight had fallen from 90 to 82 lbs. in a period of 2 to 3 months. Cervical, axillary, and inguinal nodes were enlarged, the spleen was markedly enlarged, and fluoroscopy revealed mediastinal widening. Within the next 3 months there developed a progressive anemia, and tumor masses were found in the floor of the right maxillary antrum and nasopharynx. The serum albumin was 2 and the globulin was 7 gm. per 100 cc. No Bence-Jones protein was found in the urine. Examination of the sternal marrow showed no definite abnormality. Less then 2 per cent of the total nucleated cells in the marrow were identified as plasma cells.

Biopsy of a cervical lymph node showed extensive replacement by masses of closely packed plasma cells oriented on a delicate capillary stroma. The nuclei were relatively large; whereas some had good cartwheel formation, others had prominent nuclear membranes and dispersed chromatin. Rare giant nuclei and multinucleated giant cells were seen. There were a few moderately large red-staining nucleoli, but no mitotic figures were noted.

Daily roentgen treatments were given over a period of 17 days using 135 kv. radiation filtered by 0.25 mm. Cu plus 1.0 mm. Al. Single doses of 50 r. (air) were applied to the cervical, axillary, and inguinal regions and to the spleen, and 6 $\times$ 50 r. (air)

were administered to each of two lateral portals over the nasopharynx. After a very stormy course complicated by sepsis, uremia, and coma, the patient finally recovered. Roentgenologic examination of the sinuses in January, 1947, showed no change since the examination 9 months before. No bone erosion was evident. Roentgenograms of the chest showed moderate pulmonary fibrosis relatively unaltered from earlier examinations. No abnormalities were noted in roentgenograms of the skull. In January and February, 1947, roentgen therapy (135 kv. copper filtered) was given to the mediastinum, 3 × 50 r. (air) being directed both anteriorly and posteriorly. During the same period 200 kv. irradiation (0.5 mm. Cu filter) was used over three portals directed at the maxillary and ethmoid sinuses. Improvement in the patient's condition was again noted. Roentgenograms of the spine and ribs in June, 1947, were negative. Shortly thereafter the patient developed a very severe thoracic herpes zoster. By September, 1948, the lost weight had been regained. The general improvement has been maintained to January, 1949, although the spleen is now just palpable and there is slight generalized lymph node enlargement. There is no clinical evidence of tumor of the nasopharynx or sinuses at the present time. This patient's lesion is regarded as a malignant plasma cell neoplasm involving various parts of the upper respiratory tract as well as lymph nodes and probably other tissues throughout the body.

Case 8

G. L. was a white man, 68 years old, who had had both eyes removed in 1938 for bilateral absolute glaucoma. In the next 3 years he suffered almost total loss of hearing, and in October, 1940, he began to have constant pain in the region of the right orbit. Two weeks later he noted a painful swelling at the right inner canthus. The symptoms continued and he was admitted to the Hospital of the University of Pennsylvania on January 21, 1941. Two days later the swelling at the right inner canthus began to drain purulent material. Palpation of the area revealed a firm, ovoid, 1.0 by 1.5 cm. mass, extending downward toward the innner canthus from the supra-orbital plate. A radical surgical approach revealed neoplastic tissue involving the right frontal sinus and all of the ethmoid cells on the right. Considerable bone destruction was evident. Roentgen irradiation was administered through two portals over the orbits, 2180 r. being delivered to the lesion in 26 days. The patient never returned after his last roentgen treatment and follow-up efforts were unsuccessful.

Microscopic examination of the tumor revealed broad sheets of cells on a delicate capillary stroma. The nuclei were eccentric and relatively large; they showed poor cartwheel formation, dispersed chromatin, and prominent nuclear membranes. Large red nucleoli were frequent as were giant nuclei and multinucleated cells. The lesion was interpreted as a malignant plasmacytoma.

Case 9

J. F., a white male, 72 years old, was first seen on November 13, 1946, because of complete right nasal obstruction accompanied by bleeding and discharge from the right naris. A large tumor was present which involved the nares, palate, superior alveolus, and right antrum. A specimen taken for biopsy showed large masses of plasma cells separated by ribbons of dense connective tissue. The plasma cells were arranged in sheets on a delicate capillary stroma. Nuclei were rather large with fair cartwheel formation. Multinucleated cells were present. The tumor was considered to be a malignant neoplasm. Treatment consisted of 3780 r. of high voltage radiation through multiple portals about the face and mouth. There was complete regression of the tumor with no recurrence when last seen (December, 1948).

Observations

The comparative morphologic and cytologic features of these various lesions are listed in Table I. The cases have been grouped in four categories:

1. Clinically benign plasma cell tumors of the upper respiratory tract
2. Clinically malignant plasma cell tumors of the upper respiratory tract
3. Medullary plasma cell tumors (multiple myeloma)
4. Miscellaneous inflammatory lesions

In general, it will be seen that the clinically malignant tumors of the upper respiratory tract have characteristics comparable with those found in multiple myeloma, whereas the clinically benign upper respiratory tract lesions resemble chronic inflammatory processes in their morphologic and cytologic characteristics.

Inflammatory Plasma Cell Lesions

The characteristic attributes of plasma cells found in the miscellaneous inflammatory lesions studied are:

Abundant cytoplasm
Round or polygonal cell outline
Plum-colored homogeneous cytoplasm with hematoxylin and eosin stain
Paranuclear light-staining demilune
Eccentric nucleus
Small nucleus
Round nucleus
Arrangement of chromatin in peripheral clumps with intervening clear spaces (cartwheel)
Nuclear membrane not unduly prominent
Small nucleolus, usually dark-staining, frequently absent

These features correspond to the standard descriptions[2,3] of the common plasma cell.

Maximow and Bloom[2] stated that mitotic figures in plasma cells are "exceedingly rare." We have not observed plasma cells in mitosis in lesions whose inflammatory nature seemed definite, although we have encountered them in lymph nodes in certain lesions of whose nature we are at present uncertain. Degenerative changes in plasma cells are commonly seen. These consist of accentuation of the cartwheel pattern with fading out of the cytoplasm. Eosinophilic hyaline droplets (Russell bodies) are frequently seen in the cytoplasm of plasma cells, and lying free in the tissues. Where they are present within the cytoplasm, the nucleus frequently appears large, irregular, and atypical. Binucleate plasma cells are so frequent that they may be considered a usual finding.

TABLE I

Comparative Morphologic and Cytologic Characteristics of Plasma Cell Neoplasms and Inflammatory Lesions

	Case no.	Cells in solid masses*	Replacement invasion	Multinucleate cells	Nucleus						Nucleolus		
					Size	Cartwheel	Chromatin	Membrane	Mitotic figures	Giant forms	Size	Frequency	Russell bodies
Upper respiratory tract plasma cell tumors (clinically benign)	1			Rare	Small	Good	Coarse				Small	Rare	+
	2				Small	Good	Coarse			+†	Small	Rare	+
	3			Rare	Small	Good	Coarse				Small	Rare	+
Upper respiratory tract plasma cell tumors (clinically malignant)	4	+	+	+	Large	None	Fine	Prominent		+	Large	Rare	
	5	+	+		Large	Poor	Mixed	Prominent		Rare	Medium	Many	
	6	+	+	+	Large	Fair	Coarse				Medium	Many	
	7	+	+	Rare	Large	Fair	Mixed	Prominent		Rare	Medium	Rare	
	8	+	+	+	Large	None	Fine	Prominent		+	Medium	Many	
	9	+	+	+	Large	Fair	Coarse	Prominent		+	Small	Many	
Multiple myeloma	10	+	+		Large	Poor	Fine	Prominent	+	+	Large	Many	
	11	+	+	+	Small	Poor	Fine			+	Small	Many	
	12	+	+	+	Large	Good	Coarse		+	+	Medium	Rare	
	13	+	+		Small	Good	Coarse		+	+	Large	Rare	
	14	+	+	+	Small	None	Solid			+	Large	Rare	
	15	+	+	+	Medium	Poor	Fine	Prominent		+	Large	Many	
	16	+	+		Small	Good	Coarse				Small	Many	
	17	+	+		Small	None	Fine		+		None		
	18	+	+		Small	Fair	Coarse				Large	Rare	+
Miscellaneous inflammatory lesions (composite picture)				Rare	Small	Good	Coarse				Small	Rare	+

* See text for description.

† Associated with Russell bodies only.

Although variations from the above characteristics are encountered in inflammatory lesions, they are never frequent enough to be conspicuous. In a small number of cells, the nucleus may be enlarged or the cytoplasm unusually scanty, or both of these alterations may be observed. Although the nuclei may be strikingly large at times, they do not assume the gigantic proportions seen in some of the plasma cell tumors. The nucleus occasionally varies in shape, becoming irregular or elongated. In a few cells, the cartwheel may be poorly formed, the nucleus having a prominent nuclear membrane and small chromatin clumps more evenly distributed. In rare instances a small central intranuclear body may be stained red with hematoxylin and eosin. We have interpreted this as a nucleolus. Osgood and Ashworth[4] stated that nucleoli are found in proplasmacytes but not in mature plasma cells. The paranuclear demilune is frequently not well seen. Cells having more than two nuclei may be encountered but they are rare in inflammatory lesions. Cells having more than two nuclei (which will be referred to hereafter as multinucleated cells, as distinct from binucleate cells) may be present in large numbers in certain plasma cell tumors.

In inflammatory lesions, the plasma cells are distributed throughout a tissue with a definite, although not necessarily characteristic, architecture. The plasma cells have no especial relationship to the stroma, nor do they form any particular pattern. There is no replacement of tissue by plasma cells; they merely lie within other tissues.

Plasma Cell Tumors

The characteristics listed in Table I fall into two categories. In the first group are characteristics the presence of which we believe is diagnostic of malignant plasma cell tumor and the absence of which makes malignancy unlikely. These diagnostic features are: (1) orientation of the plasma cells in broad sheets on a delicate stroma consisting largely of capillaries, and (2) replacement of other tissue by such plasma cell sheets, as opposed to the disposition of plasma cells throughout another tissue. All of the clinically malignant plasma tumors in our series show these two characteristics (Fig. 1), whereas none of the clinically benign lesions show either one (Fig. 2).

This difference in pattern is particularly well brought out by the use of silver stains. In the malignant lesions the cells are closely packed, with a few delicate reticulin fibers dividing them into large groups. The reticulin fibers usually run approximately parallel (Fig. 3). In the benign lesions, the silvered fibers are coarse, numerous, and irregularly disposed, dividing loosely lying plasma cells into small groups or even

surrounding single cells (Fig. 4). The fiber distribution is essentially that of the background tissue and appears unrelated to the plasma cells themselves.

In the second group are those characteristics which, when present in significant degree, are strongly suggestive of malignancy, but which when absent do not rule out malignant tumor. Indeed, one cannot safely consider malignancy unlikely merely because these features are absent. These characteristics include alterations in nuclear-cytoplasmic ratio and nuclear characteristics, and multinucleated cells. It has been noted previously that the plasma cells of benign (or inflammatory) lesions may show a few cells with increased nuclear-cytoplasmic ratio, or poor cartwheel formation with prominent nuclear membrane and dispersed chromatin; rarely strikingly large nuclei also may be seen. If the majority of cells of a plasma cell tumor show increased nuclear-cytoplasmic ratio or poor cartwheel formation, or if a sizable percentage of nuclei are greatly enlarged, it is probable that the tumor is malignant (Fig. 5). The presence of mitotic figures in plasma cells is a further point indicating a probable malignant nature.

In inflammatory lesions and benign tumors, plasma cells may occasionally have a small, central, chromatin clump having the appearance of a nucleolus; in rare instances this may stain red with hematoxylin and eosin. Large nucleoli, usually red staining, have been noted only in the group of malignant tumors (Fig. 6).

Multinucleated plasma cells (having more than two nuclei) are extremely rare in inflammatory lesions and benign tumors. In many of the malignant tumors of this series they were present in large numbers (Fig. 5).

Russell bodies are common in inflammatory lesions and benign tumors. We did not note their presence in any of the malignant tumors of the upper respiratory tract in this series, although we have encountered them, at times in great numbers, in cases of multiple myeloma. Bayrd[5] has reported their presence in this disease. We have not regarded the absence of Russell bodies as a reliable criterion of malignancy. Frequently, inflammatory cells other than plasma cells are seen throughout benign plasma cell tumors, as well as in miscellaneous inflammatory lesions. In the malignant plasma cell tumors, however, mixed inflammatory cells are seen only in association with ulceration.

The characteristics listed in this second group are essentially those of malignant tumors in general. It is again emphasized that the absence of cytologic criteria of malignancy is not evidence of the benign nature of a plasma cell tumor. The absence of the specific pattern described

in the first category above, however, makes it extremely unlikely that the lesion lacking it is malignant.

Discussion

Hellwig[1] pointed out that where the usual cytologic criteria of malignancy are present, there is little difficulty in recognizing a plasma cell tumor as malignant. The confusion arises in those cases in which the tumor cells closely resemble normal plasma cells. Our study bears out the statement that malignant plasma cell tumors may not have the usual cytologic characteristics of malignancy. When such characteristics are present, a diagnosis of malignant tumor can be made; when they are absent, however, malignancy cannot be excluded. This study leads us to believe that the most reliable guides to the nature of a plasma cell tumor are: (1) the presence of broad sheets of cells oriented on a delicate vascular stroma, and, (2) replacement of tissue by tumor cells rather than infiltration of tissue. If these features are absent, it is unlikely that the lesion is malignant. If we apply these criteria to the lesions illustrated by Lumb and Prossor,[6] the same correlation between histologic features and clinical course is noted in their material as in our series. Although Bayrd[5] found a correlation between purely cytologic features and prognosis in his cases of multiple myeloma, no consistent relationship of this sort could be demonstrated in our series of plasma cell tumors of the upper respiratory tract.

The close resemblance of clinically malignant plasma cell tumors of the upper respiratory tract to multiple myeloma suggests a relationship between the two conditions. The marked tendency toward multiple bone involvement associated with tumors of the respiratory tract further supports such a relationship. Of the six tumors of the upper respiratory tract considered to be malignant, one was ultimately associated with widespread bone and soft tissue involvement; one case showed generalized, and one regional, lymph node involvement. All of these malignant plasmacytomas involved multiple areas in the upper respiratory tract by invasive growth. In 2 cases bone was involved in the locally destructive process. In contrast, the clinically benign plasma cell lesions were small, well localized, and non-invasive.

The close resemblance of the clinically benign extramedullary plasma cell tumors to chronic inflammatory lesions suggests that they too may be basically inflammatory, and raises a question as to the existence of a benign plasma cell neoplasm.

The age distribution of the malignant plasma cell tumors is quite distinct from that of the benign lesions (Table II). The ages at onset

TABLE II

Clinical Features of Plasma Cell Tumors of the Upper Respiratory Tract

Case no.	Age	Sex	Site of lesion	Clinical features	Surgery	Radiation	Result	Period of observation
1 (H. G.)	43	M	Mastoid	Small local lesion	Extirpation once	None	No recurrence	4 years
2 (F. F.)	45	M	Gingiva	Small local lesion	Extirpation once	None	No recurrence	2 years
3 (O. G.)	33	M	Maxillary sinus	Small local lesion	Extirpation once	None	No recurrence	3 years
4 (W. C.)	52	M	Nasal cavity	Large local lesion; distant bone and soft tissue metastases	Attempted removal twice; recurred in 1 year	X-rays and radium to area of origin and multiple metastases; also radioactive phosphorus tracer study and one course of nitrogen mustard	Local lesion controlled; patient living, with extensive metastases	3 years
5 (M. H.)	83	F	Nasal cavity	Large local lesion	Biopsy only	X-rays to local lesion	Local lesion controlled; no metastases	3 years
6 (L. S.)	73	M	Nasal cavity	Large local lesion; cervical node metastases	Local removal and neck dissection; lesion recurred	Radium to nasal passages	Local lesion controlled; no metastases	2 years
7 (E. C.)	59	F	Nasopharynx	Extensive lesion; generalized lymph node involvement; A/G ratio reversed	Removal attempted twice; lesion recurred	X-rays to nasopharynx, paranasal sinuses, lymph node areas, and mediastinum	Local lesion controlled; lymphadenopathy greatly reduced	3 years
8 (G. L.)	68	M	Frontal and ethmoid sinuses	Large local lesion; bone invaded	Partially removed	X-rays to local lesion	Unknown	Lost to follow-up
9 (J. F.)	72	M	Nasal cavity, gingiva, maxillary sinus	Large local lesion	Biopsy only	X-rays to regions involved	Local lesion controlled	2 years

of the patients with malignant plasmacytomas ranged from 50 to 83, with an average of 66, a distribution paralleling that of multiple myeloma.[7] The benign lesions, on the other hand, occurred in a considerably younger age group, first being noted by the patients at ages ranging from 33 to 45.

A finding worthy of comment is the high incidence of malignant plasma cell tumors in the maxillary sinus. In the series of Figi, Broders, and Haven,[8] and of Lumb and Prossor,[6] all plasma cell tumors in this location proved to be malignant. However, in one of the 2 cases in this series in which the antrum was involved, we regard the tumor as benign. Tumors of other locations in the upper respiratory and food passages vary considerably in their malignant potentialities in all three series.

Reference to Table II shows that all plasma cell lesions of the upper respiratory tract which were considered to be benign were eradicated by surgical excision alone, and showed no tendency to recur. On the other hand, 3 malignant tumors treated primarily by surgical means recurred locally within 1 year; 2 recurred twice, and the third recurred three times. However, a remarkable response to radiation was shown by the tumors of this group. Three of the malignant plasmacytomas were treated by radiation primarily; the other 3 only after recurrence had followed repeated attempts at surgical removal. The result is unknown in one case but the remaining 5 were controlled by this means, 4 of the tumors disappearing completely. This remarkable radiosensitivity was noted by Ringertz[9] in his study of nasopharyngeal tumors. In their response to radiation the malignant plasma cell tumors have much in common with the malignant lymphoid tumors. It must be pointed out, however, that, whereas radiation therapy may have some beneficial effect on metastatic soft tissue and lymph node lesions, its application to the primary tumor does not necessarily prevent dissemination (case 4). Our experience indicates that, whereas simple surgical excision constitutes adequate treatment for the benign lesions, radiation therapy is of particular value in the treatment of the malignant tumors. This difference in therapeutic response makes accurate histologic evaluation all the more important.

Summary

The histologic characteristics of 9 cases of plasma cell tumor involving the upper respiratory and food passages (6 malignant neoplasms and 3 benign or inflammatory lesions) have been studied and compared with the histologic picture of 9 cases of multiple myeloma and with a

variety of inflammatory lesions in which plasma cells were an especially conspicuous feature.

The replacement of tissue by broad sheets of plasma cells oriented on a delicate capillary stroma is indicative of a malignant plasma cell tumor. The absence of this pattern makes it unlikely that a plasma cell tumor is malignant.

The usual cytologic criteria of malignancy are reliable in the study of plasma cell tumors when they are present in considerable degree. The absence of these criteria, however, does not exclude malignancy.

Benign plasma cell lesions of the upper respiratory tract may be adequately treated by surgical excision. The malignant plasma cell neoplasms respond well to radiotherapy; in the cases reported, recurrence was the rule in cases treated by surgical removal.

We wish to express our gratitude to Drs. Eugene P. Pendergrass, Thomas Fitz-Hugh, Jr., Harry P. Schenck, Karl M. Houser, Gabriel Tucker, and William Hewson for their generosity in making clinical records available.

REFERENCES

1. Hellwig, C. A. Extramedullary plasma cell tumors as observed in various locations. *Arch. Path.*, 1943, **36**, 95–111.
2. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1935, ed. 2, 662 pp.
3. Marschalko. Cited by Hellwig.[1]
4. Osgood, E. E., and Ashworth, C. M. Atlas of Hematology. J. W. Stacey, Inc., San Francisco, 1937, 255 pp.
5. Bayrd, E. D. The bone marrow on sternal aspiration in multiple myeloma. *Blood*, 1948, **3**, 987–1018.
6. Lumb, G., and Prossor, T. M. Plasma cell tumours. *J. Bone & Joint Surg.*, 1948, **30-B**, 124–152.
7. Lichtenstein, L., and Jaffe, H. L. Multiple myeloma. A survey based on 35 cases, 18 of which came to autopsy. *Arch. Path.*, 1947, **44**, 207–246.
8. Figi, F. A., Broders, A. C., and Havens, F. Z. Plasma cell tumors of the upper part of the respiratory tract. *Ann. Otol., Rhin. & Laryng.*, 1945, **54**, 283–297.
9. Ringertz, N. Pathology of malignant tumors arising in the nasal and paranasal cavities and maxilla. *Acta oto-laryng.*, 1938, Suppl. 27, 405 pp. (Cited by Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1940, ed. 4.)

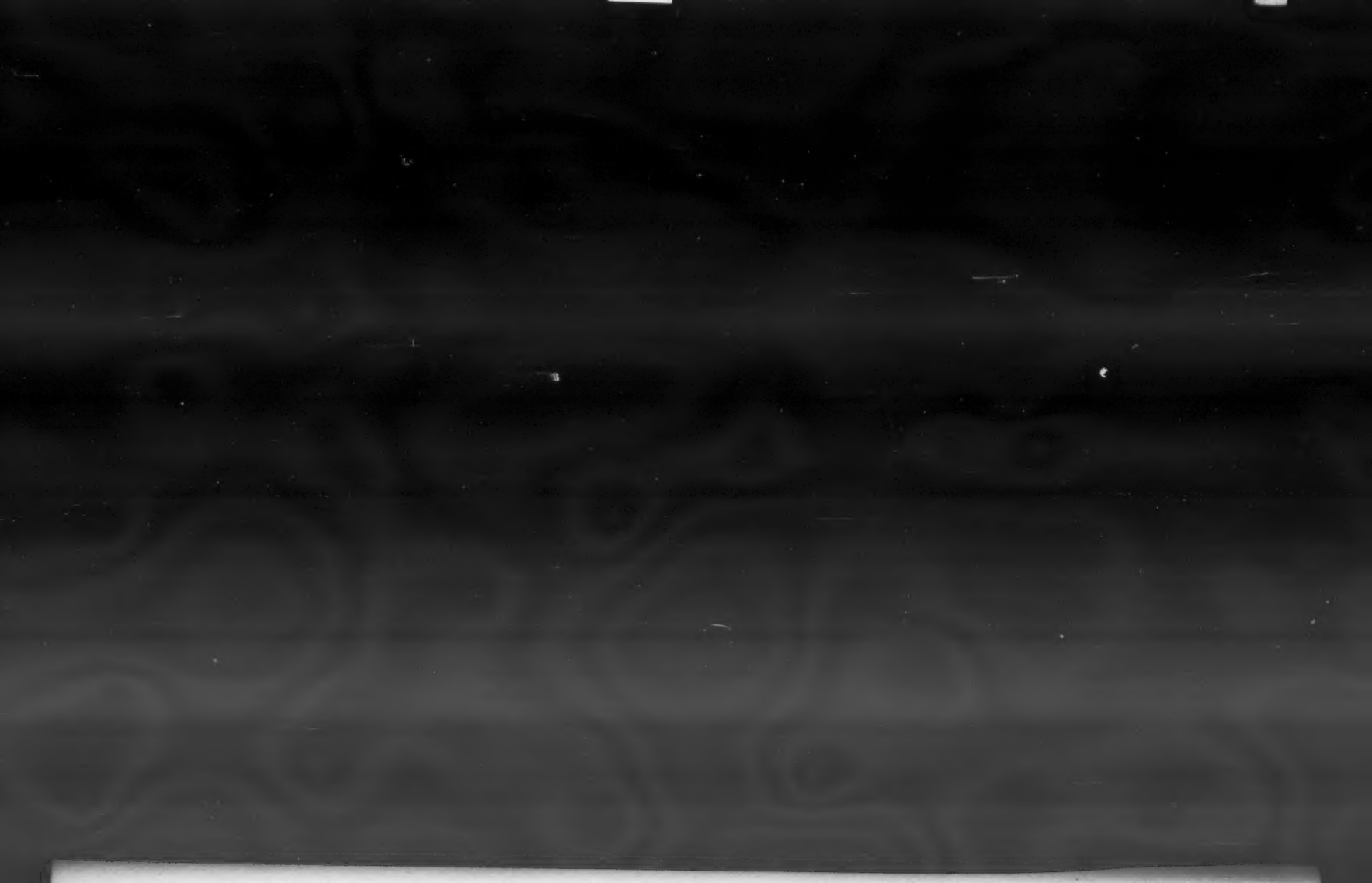
[*Illustrations follow*]

DESCRIPTION OF PLATES

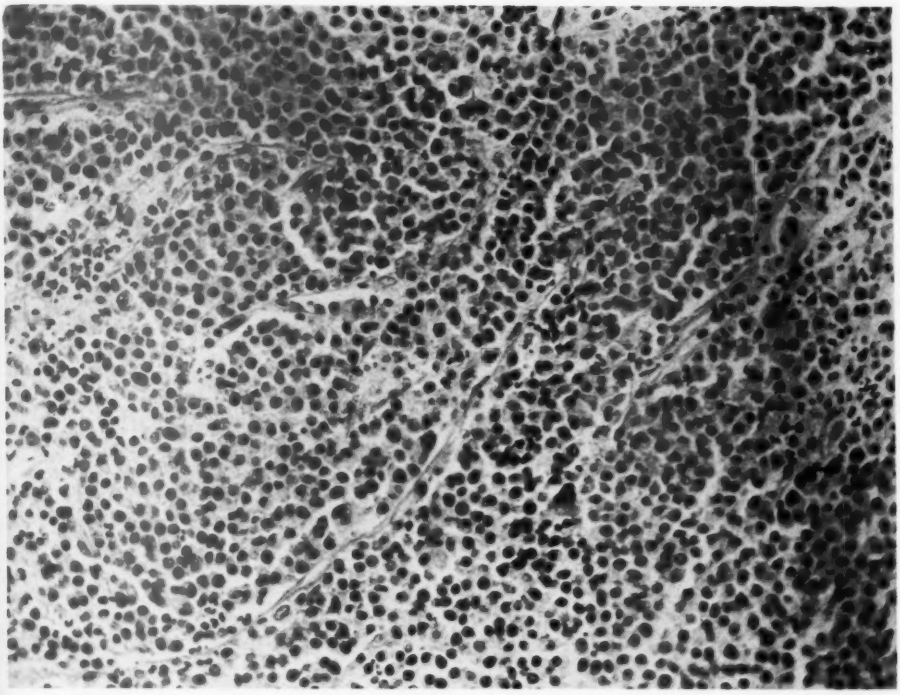
PLATE 67

FIG. 1. Case 4 (W. C.). Malignant plasma cell tumor of nasal cavity. The tumor cells are closely packed in broad sheets, and are disposed in a definite pattern, being oriented on a delicate capillary stroma. Hematoxylin and eosin stain. × 650.

FIG. 2. Case 3 (O. G.). Benign plasma cell lesion of antrum. Plasma cells are loosely disposed without pattern throughout a tissue matrix. A Russell body is present near the lower left-hand corner. Hematoxylin and eosin stain. × 280.



1



2

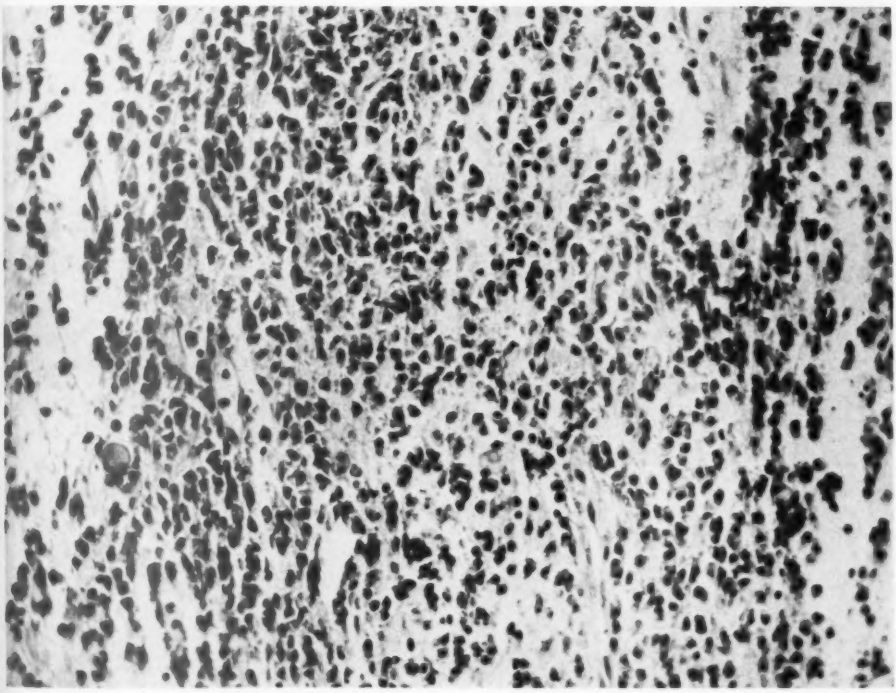


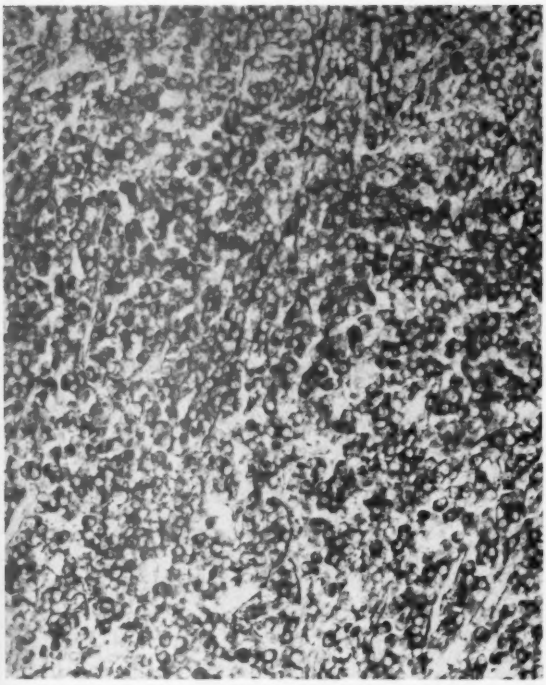
PLATE 68

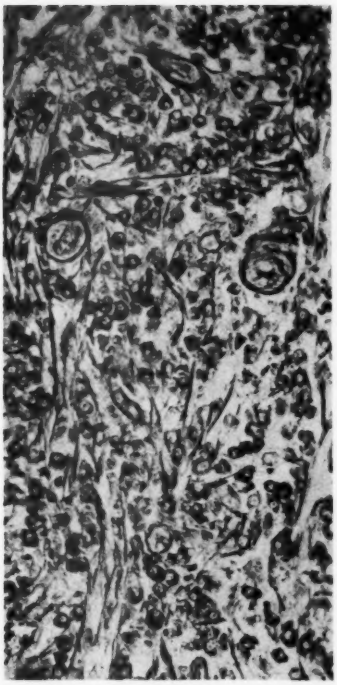
FIG. 3. Case 17. Multiple myeloma. Fine reticulin fibers, oriented in more or less parallel fashion, separate masses of tumor cells. Laidlaw's stain. × 280.

FIG. 4. Case 3 (O. G.). Benign plasma cell lesion of maxillary antrum. Coarse reticulin fibers run haphazardly. Small groups of plasma cells lie between them without uniform relationship. Laidlaw's stain. × 280.

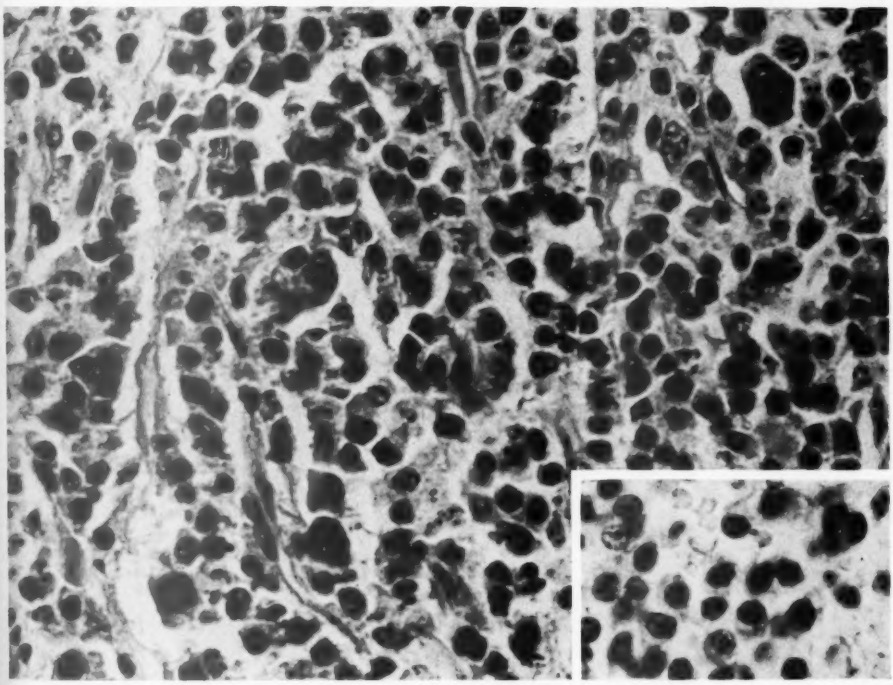
FIG. 5. Case 13. Multiple myeloma and plasma cell leukemia. The cytologic features of malignancy are conspicuous. The nuclei are strikingly large with relation to the cytoplasm; there is great variability in "cartwheel" appearance; gigantic nuclei and multinucleated cells are numerous. Hematoxylin and eosin stain. × 650.

FIG. 6. Case 16. Multiple myeloma. The photomicrograph shows numerous large nucleoli. These were stained red with hematoxylin and eosin. × 650.





4



6

BENIGN LYMPHOMA OF RECTUM*

ELWYN L. HELLER, M.D., and HOMER H. LEWIS, JR., M.D.
(From the Department of Pathology and the Department of Surgery, University of Pittsburgh, Pittsburgh 13, Pa.)

The benign lymphoid tumor of the rectum was first described in 1890 by Shattock.[1] Of the scattered reports in the literature since that time,[2-20] relatively few have been in the American literature.† The importance of the lesion lies in the recognition of its benign characteristics in spite of histologic features simulating malignancy.

We employ the term "lymphoma" because we regard the lesion as a lymphoid tumor and qualify it with "benign" because of the frequency with which "lymphoma" is applied to malignant lymphoid tumors. The lesion also has been designated lymphadenoma, lymphadenoid polyp, and lymphoid polyp.

REPORT OF CASES

Case 1

K. P., a woman, 58 years old, complained of rectal discomfort intensified by defecation, and slight rectal bleeding. Rectal examination revealed hemorrhoids and a polyp 1.5 cm. in diameter attached by a narrow pedicle to the mucosa above the internal hemorrhoids. Hemorrhoidectomy and excision of the polyp were done. Follow-up examination 15 months later revealed no evidence of recurrence of the lymphoid tumor.

Case 2

V. S. was a woman, 30 years of age, who had complained of episodes of diarrhea over a period of 3 years. Routine proctoscopic examination disclosed a polyp 2 cm. in diameter attached anteriorly at the pectinate line. Follow-up examination 9 months subsequent to excision of the polyp revealed no recurrence. The symptoms of colitis had persisted.

Case 3

M. B., a woman, 36 years old, complained of slight rectal bleeding and protrusion of a mass into the anus. A polyp 1.5 cm. in diameter was excised from the right lateral rectal wall. There was no recurrence at follow-up examination 8 months later.

Case 4

J. M. was a woman, 24 years old, who was admitted for a minor gynecologic operation, during the course of which a rectal polyp 1 cm. in diameter was excised. It was attached to the left posterior wall of the rectum. The patient had no symptoms referable to the rectal lesion. Follow-up examination 5 months later revealed no recurrence.

* Presented at the Forty-sixth Annual Meeting of The American Association of Pathologists and Bacteriologists, Boston, April 16, 1949.
Received for publication, April 27, 1949.

† The earliest cases recorded in the American literature are briefly described in a footnote in the publication by Stout.[7]

Case 5

E. C., a woman, 41 years old, complained of moderately severe rectal pain and slight bleeding during defecation. Rectal examination revealed an ovoid submucosal tumor mass 5 cm. in diameter in the rectal wall posteriorly. The mass was excised and the wound healed readily. There was no recurrence at follow-up examination 2 months later.

Case 6

The patient was a woman, 37 years old, who complained of slight intermittent rectal bleeding and a sensation of incomplete evacuation following defecation. A nodular mass, 3 by 3 by 2 cm. with a broad base, was excised from the posterior rectal wall immediately above the sphincter. 1400 mg. hours of radium were given following excision. Insufficient time has elapsed for follow-up examination.

Case 7

M. Y., a woman, 28 years of age, had been constipated all her life and had bleeding at stool for 3 weeks prior to excision of a submucous benign lymphoma that measured 2 cm. in diameter.

Case 8

M. W. was a woman, 42 years old, who complained of constipation and a continuous sensation of a mass in the rectum. There had been occasional rectal bleeding during the preceding year. A polyp 1.5 cm. in diameter was excised from the rectum.

Case 9

A. G., a woman, 45 years of age, complained of rectal bleeding and slight pain occurring 6 months after a hemorrhoidectomy. A benign lymphoma 8 by 7 by 3 mm. was excised.

Clinical Features

The tumor occurs in both sexes and at all ages. Three reported cases have occurred in children under 10 years of age. Local symptoms may result from lesions unrelated to the benign lymphoma, the detection of which is incidental to routine rectal examination. At times rectal pain, slight bleeding, or protrusion of the mass into the anal canal occur. Significant is the fact that in none of the cases has there been evidence of disease elsewhere in the lymphatic tissues.

The majority of the lesions are polypoid, but intramural nodulation is noted occasionally. In the latter type the induration may lead to the clinical impression of malignancy, and cases have been treated by irradiation or surgical resection of the rectum under the mistaken belief that the lesion was malignant. The tumors vary from a few millimeters to 8 cm. in diameter. The lesion is usually solitary, the mucous membrane is intact, and there is no site of predilection in the rectum. The lesion has been reported in the colon above the rectum.[7,18]

Pathologic Features

Grossly, the lesion is nodular and sharply circumscribed. On section a solid, glistening, translucent fleshy tissue is noted.

Microscopically, the rectal mucosa overlying the lesion usually shows pressure atrophy. In many cases the mucosal stroma is heavily infiltrated with lymphocytes which are directly contiguous with the lymphoid mass of the submucosa. The infiltration may replace the muscularis mucosae. The main lymphoid mass is immediately submucosal.

On casual inspection the mass resembles a normal lymph node in having numerous lymphoid follicles with germinal centers. The interfollicular tissue is composed of packed lymphocytes and scattered reticular cells in a delicate meshwork of reticulum fibers. At times the lymphoblastic cells show numerous mitotic figures and may be dispersed through the interfollicular areas beyond the confines of the germinal centers. Sinusoids are not present. At the periphery of the lymphoid mass no capsule is noted, although the border between lymphoid and adjacent connective tissue is fairly sharp. Small foci of lymphocytes are scattered beyond the limits of the main lesion.

Discussion

We concur with the general interpretation that the lesion is a benign lymphoid neoplasm. The character of the proliferation is not that of an inflammatory process. Evidence of infectious or inflammatory reaction of adjacent areas is lacking. The size and sharp circumscription of the lesion are at variance with the usual inflammatory lesions of the colonic mucosa. The intact mucosa over the lesion excludes the ulcerative type of inflammatory granuloma.

In particular there are three features which simulate malignant proliferation. These are discussed in the succeeding paragraphs.

Destruction of the Muscularis Mucosae. The destruction of the muscularis mucosae is regarded as purely a pressure phenomenon from a slowly expanding lesion of the underlying submucosa. We have observed this phenomenon in non-tumorous reactions. The character of the peripheral expansion and the type of tissue involved lead to histologic patterns simulating malignant growth.

Absence of a Capsule. Proliferative lesions of lymphatic tissue generally involve lymphoid tissue which normally possesses a distinct capsule. In such cases infiltration and destruction, or absence, of a capsule has considerable significance in the determination of malignancy. The lymphoid tissue of the colonic mucosa which gives origin to the lesion under discussion possesses no capsule under normal conditions so its absence in growth disorders does not have equivalent significance.

Diffusion of Lymphoblastic Cells Beyond the Confines of the Follicles. In 2 of our cases lymphoblastic cells were noted in the interfollicular

areas and mitotic figures were noted with disturbing frequency. However, the follicles were readily discernible and the pattern of growth was not that conforming to any of the several well established types of malignant lymphoid tumors.

The clinical behavior of these tumors is of utmost importance in establishing their benign nature. None of our cases has shown signs of recurrence following local excision. It is significant that in the literature there are no reports of recurrence and many cases have had prolonged follow-up study.

It appears that benign lymphomas of the rectum are relatively common. In the material of the Department of Surgical Pathology of the Presbyterian and Woman's Hospitals in Pittsburgh, benign lymphomas constitute 3 per cent of 259 anorectal tumors encountered during the past 4½ years. Ehrlich and Hunter[16] reported a 14.6 per cent incidence of lymphoid polyps in 363 tumors of rectum and anus. The 6 cases reported by Dick[10] represented 9 per cent of 70 benign polyps collected over a 20-year period.

Summary

Nine cases of benign lymphoma of the rectum form the basis of this report. The lesion presents pathologic features which simulate malignancy, but on analysis, these features are found to be otherwise interpretable. The clinical course establishes the benign character of these lymphomas. The lesion is more common than the infrequent references to it would indicate.

REFERENCES

1. Shattock, S. G. Polypi of lymphatic tissue from a child's rectum. *Tr. Path. Soc. London*, 1890, **41**, 137–139.
2. Ball, C. B. The benign neoplasms of the rectum. *Brit. M. J.*, 1890, **2**, 1353–1355.
3. Greig, D. M. Lymphoma of the rectum. *J. Path. & Bact.*, 1908–09, **13**, 49–50.
4. Bérard, L., and Chalier, A. Le lymphadénome du rectum. *Lyon chir.*, 1909, **2**, 146–153.
5. Lehmann, H. Unter dem Bilde der "Polyposis" in Erscheinung tretende, umschriebene Hyperplasie des lymphatischen Gewebes im Dickdarm. *Deutsche Ztschr. f. Chir.*, 1925, **190**, 391–396.
6. Knoflach, J. G. Ein Beitrag zur Pathologie und Chirurgie der Mastdarmpolypen. *Wien. klin. Wchnschr.*, 1927, **40**, 876–877.
7. Stout, A. P. Isolated lymphoid hyperplasia in the cecum and appendix of children. *Am. J. Dis. Child.*, 1927, **34**, 797–806.
8. Siemens, W. Lymphadenoider Polyp des Rektums. *Zentralbl. f. Chir.*, 1931, **58**, 2070–2072.
9. Konjetzny, G. E. Lymphadenoide Polypen des Rektum. *Zentralbl. f. Chir.*, 1931, **58**, 3154.

10. Dick, W. Lymphadenoide Rektumpolypen. *Beitr. z. klin. Chir.*, 1932, **156**, 263–274.

11. Lévy, M., and Armingeat, J. Tumeur infiltrée à lymphoblastes de la paroi rectale. *Arch. d. mal. de l'app. digestif*, 1933, **23**, 767–771.

12. Epstein, A. A. Über Lymphadenome der Rectum. *Arch. f. klin. Chir.*, 1933, **175**, 351–356.

13. Dukes, C. Lymphoma of the rectum. *Proc. Roy. Soc. Med.*, 1934, **27**, 926–927.

14. Hayes, H. T., Burr, H. B., and Pruit, L. T. Lymphoid tumors of the colon and rectum. *Surgery*, 1940, **7**, 540–545.

15. Smith, T. E. Primary lymphoid tumors of the rectum resembling internal hemorrhoids. *J. A. M. A.*, 1943, **121**, 495–498.

16. Ehrlich, J. C., and Hunter, O. B., Jr. Tumors of the gastrointestinal tract. A survey of 813 in persons of military age during World War II. *Surg., Gynec. & Obst.*, 1947, **85**, 98–106.

17. Miller, D. Benign lymphoma of the rectum. *Am. J. Surg.*, 1948, **76**, 769.

18. Gruenwald, P. Abnormal accumulations of lymph follicles in the digestive tract. *Am. J. M. Sc.*, 1942, **203**, 823–829.

19. Li, I. Y. Benign lymphoma of the rectum. *Surgery*, 1948, **23**, 814–820.

20. Scarborough, R. A., and Klein, R. R. Polypoid lesions of the colon and rectum. *Am. J. Surg.*, 1948, **76**, 723–727.

[*Illustrations follow*]

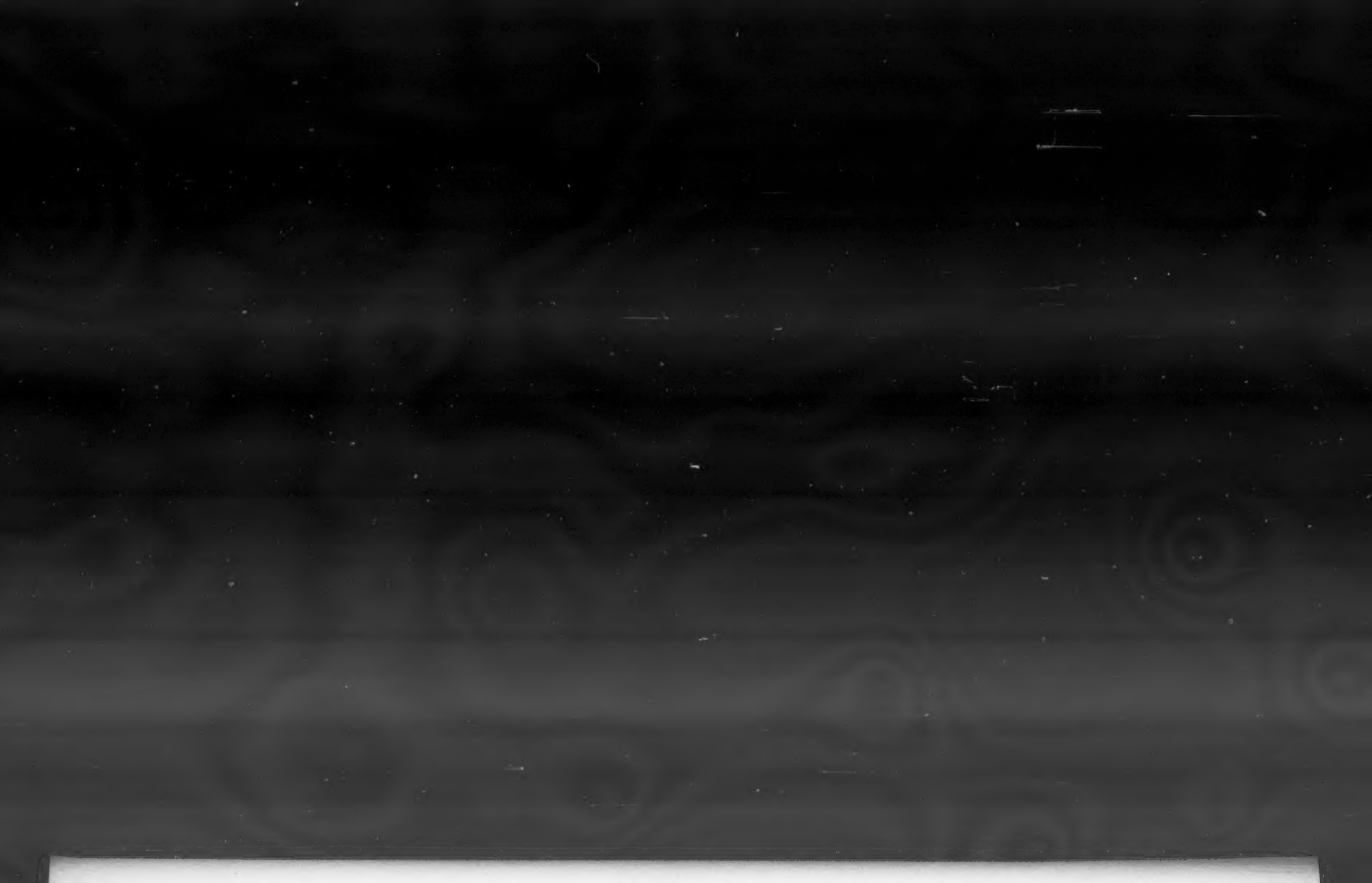
DESCRIPTION OF PLATES

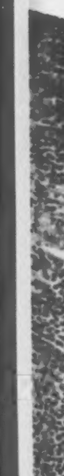
PLATE 69

FIG. 1. Benign lymphoma of rectum. The central lymphoid mass and the polypoid contour of the lesion at low magnification. × 15.

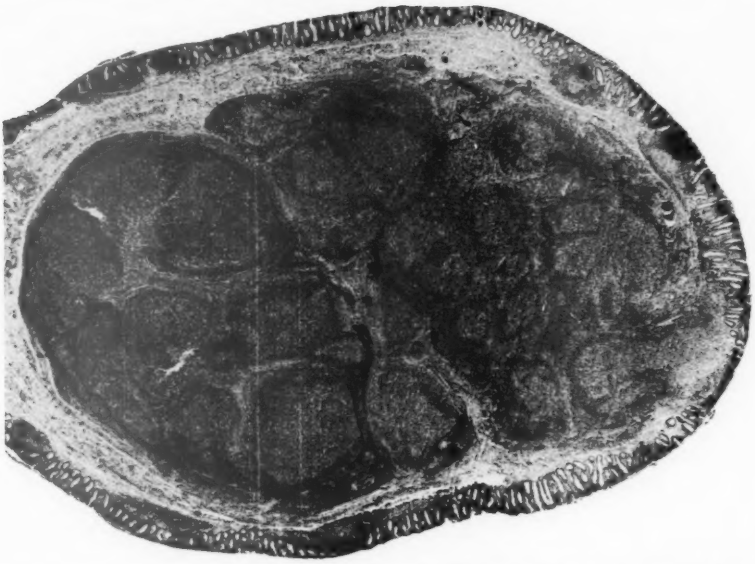
FIG. 2. The expanding submucosal lymphoid mass replaces the muscularis mucosae, slender fasciculi of which are still visible. × 150.

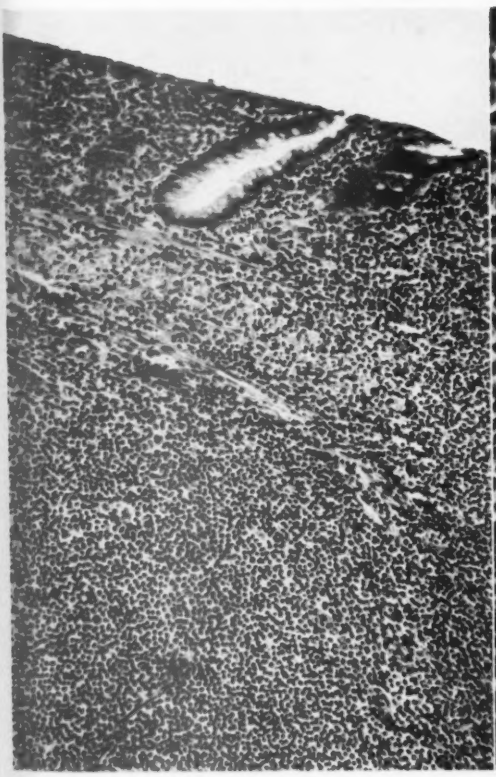
FIG. 3. The predominating structure of the lesion, showing mature lymphocytic cells with sparse stroma. × 375.



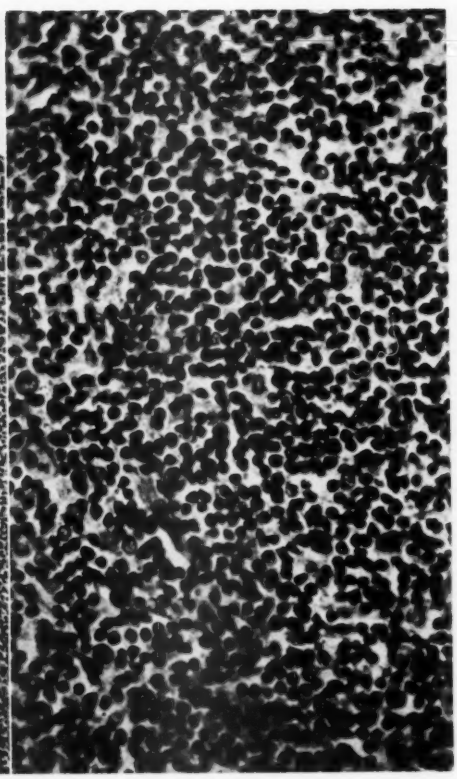


1





2



3

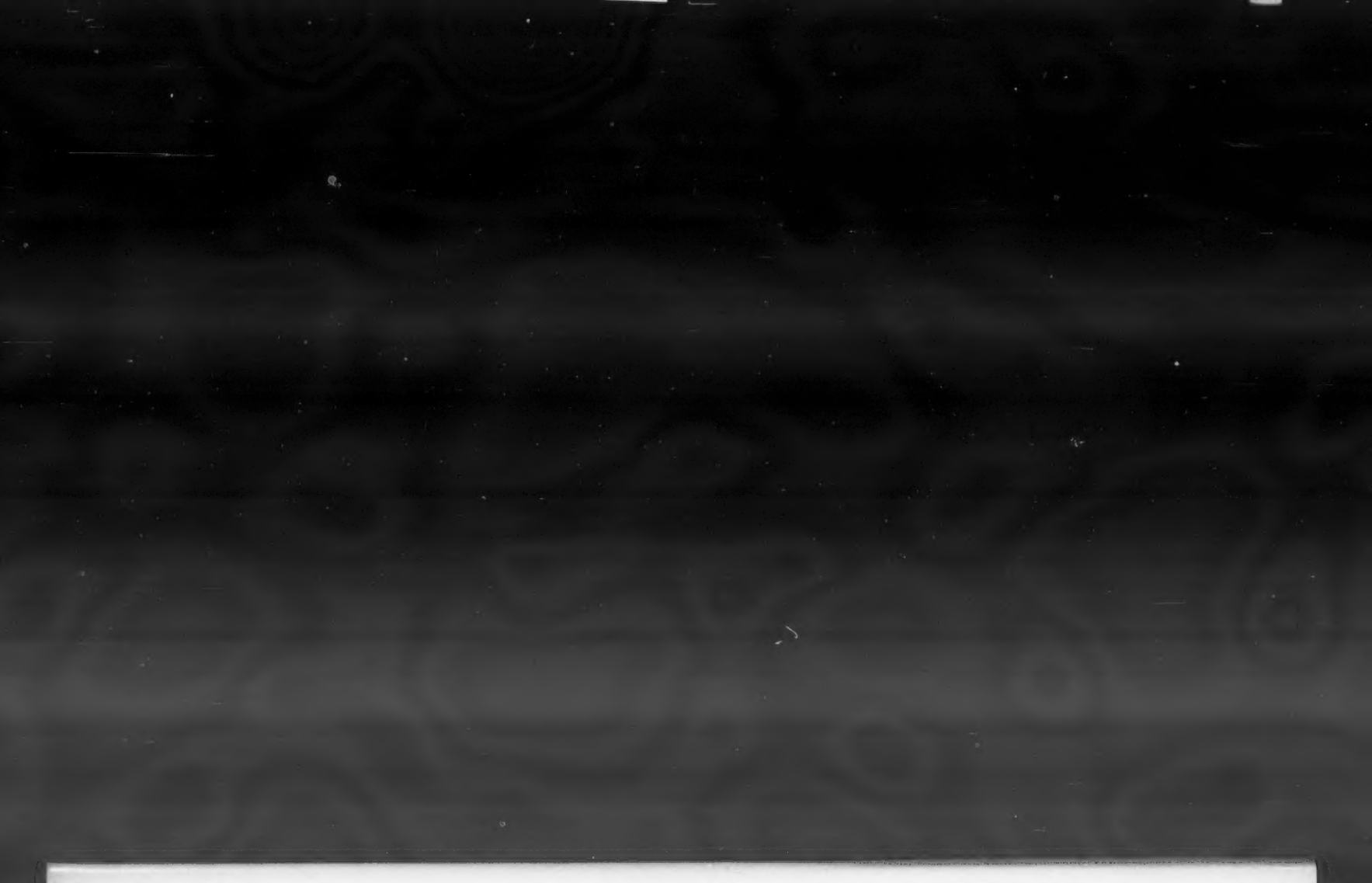
Heller and Lewis

Benign Lymphoma of Rectum

PLATE 70

FIG. 4. Benign lymphoma of rectum. The edge of the submucosal lymphoid mass appears at the left. There is no capsule and lymphoid cells diffuse peripherally. × 150.

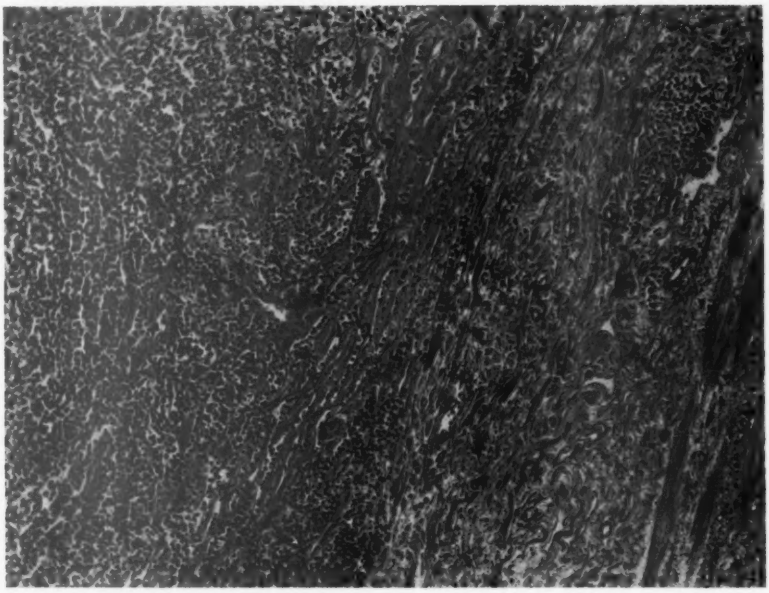
FIG. 5. Lymphoblastic cells dispersed beyond the germinal centers of the lymphoid follicles. × 375.



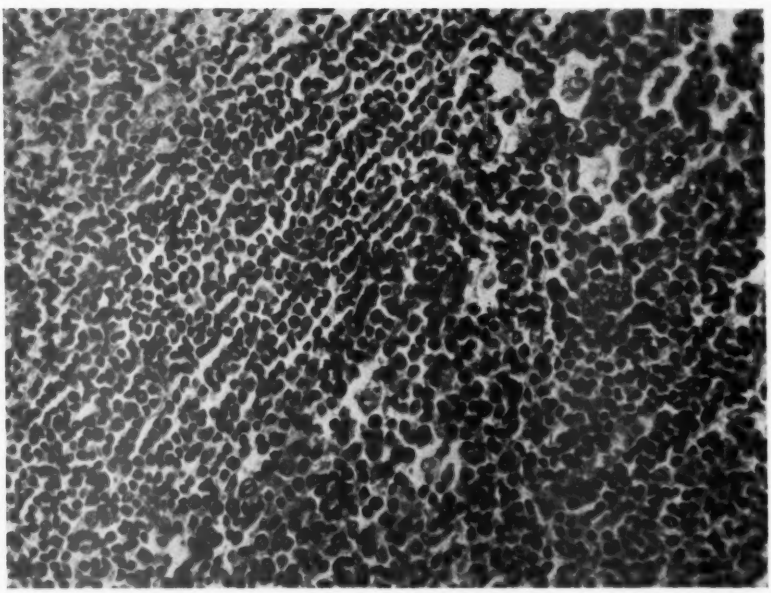
A

H

4



5



Heller and Lewis Benign Lymphoma of Rectum

PLEURAL MESOTHELIOMA *

WILLIAM N. CAMPBELL, M.D.
(From the Department of Pathology, Temple University Medical School, Philadelphia, Pa.)

The diagnosis of pleural mesothelioma was once held in ill repute by pathologists. The failure to accept pleural mesothelioma as a distinct entity was due mainly to a lack of understanding of the neoplastic multipotentialities of the mesothelial cells of the pleura. In addition, many so-called primary pleural tumors were shown by careful gross and cytologic scrutiny to have been metastatic from somewhere else in the body, the usual primary site being the bronchus. As a consequence it is probable that many true pleural mesotheliomas have been unrecognized, pathologists being overcautious in making such a diagnosis. Klemperer and Rabin,[1] and Saccone and Coblenz[2] have done much to establish "pleural mesothelioma" as a distinct clinicopathologic entity. The sex and age incidence, clinical course, diagnostic aspects, and gross characteristics have been well described.[1,2]

The right and left pleurae are affected with equal frequency, and the tumor is about twice as common in males as females.[2] Although pleural mesotheliomas may involve any age group, the greatest incidence occurs between 40 and 60 years of age.[2]

Clinically, the patient with a pleural mesothelioma usually has a rapid downhill course terminating in death. Pleuritic chest pain, cough, and dyspnea are usually prominent symptoms. Unilateral serous or serosanguineous pleural effusion that rapidly recurs after aspiration is typical. Aspiration of this fluid does little to relieve the dyspnea. Differential diagnosis is mainly from tuberculosis or from metastatic tumor of the pleura. Radiologic examination,[3] cytologic study of aspirated fluid,[4] or needle biopsy of the involved pleura[5] may aid in diagnosis.

At autopsy a thick, firm, grayish tumor mass is found that completely encases or surrounds the involved lung, with little or no evidence of parenchymal invasion. This gross appearance is said to be of more diagnostic importance than the histopathologic features of the tumor.[2]

The histopathologic aspects and behavior of this tumor are little appreciated. Saccone and Coblenz[2] stated that microscopically the majority of these tumors showed an epithelium-like pattern, being composed of acinar structures which were lined by a single layer of polyhedral cells. Klemperer and Rabin[1] concluded that pleural mesothe-

* Submitted to the Faculty of the School of Medicine of Temple University in partial fulfillment of the requirements for the degree of Master of Science in Pathology.
Received for publication, June 24, 1949.

liomas might "present the characteristics of epithelium, of connective tissue or of both." They reported a case having both epithelial and mesenchymal features and mentioned several similar cases found in the literature. Stout and Murray[6] mentioned that both carcinomatous and sarcomatous qualities are found in the same tumor. Zeckwer[7] recognized the pleomorphic tendencies of these growths, believing the pleomorphism to be characteristic. However, Saccone and Coblenz[2] quoted Scheidegger[8] as noting that "nothing that is characteristic for these pleural tumors can be deduced from the histologic picture." This latter statement seems to be the concensus among most authors. Little is mentioned in the literature about the behavior and modes of metastasis of the pleural mesothelioma. Vitkus[9] has claimed that metastases are exceedingly rare. Others[2] have stated that usually there is regional lymph node metastasis and that occasionally metastasis occurs to distant parenchymatous organs, presumably by the blood stream. It has been stated that the tumor occasionally invades the peritoneum.[6] Saccone and Coblenz[2] postulated spread by pleural fluid movements.

Cases of pleural mesothelioma have been reported from many clinics, but because of the rarity of the lesion the number of reported cases from any one clinic always has been exceedingly small. (Since 1941, the largest number of verified cases reported by any single group has been 2.) In 1943 Saccone and Coblenz[2] carefully surveyed the literature and found about 200 cases which they believed presented the gross and cytologic characteristics of pleural mesothelioma. Since then 18 cases of pleural mesothelioma (or "endothelioma") have been reported by 13 different authors in the English literature.[2-6,10-18] During the past 8 years, 4 of a total of 3533 consecutive autopsies performed at Temple University Hospital have fulfilled the necessary criteria for the diagnosis of pleural mesothelioma. In reviewing our 4 cases (a relatively large series to be reported by any single group), rather constant cyto-architecture and behavior were noted which appear to have been neglected in the literature. Because of this it was considered timely to review the cases in the recent literature to ascertain if the tenets suggested by our own 4 cases were sound. (Because of the difficulties attendant in evaluating the older literature,[2] emphasis is placed upon more recent reports and reviews wherein gross findings, cytologic descriptions, and illustrations are more adequate and accurate.) Our suppositions as to cellular features and behavior have been borne out.

The criteria used for selecting cases of pleural mesothelioma were a gross picture of a firm pleural mass encasing the involved lung, a cyto-architecture compatible with that previously described for pleural meso-

thelioma[1,2] and, most important, the lack of a demonstrable primary tumor in the lung or elsewhere in the body. For obvious reasons, only autopsied cases were used in this study. Eight of the 18 recently reported cases are not included. The second case reported by Weissman[3] was not autopsied. Four cases[6,10,18] were not considered to fulfill the gross and cytologic criteria necessary for diagnosis. The 3 cases reported by Doub and Jones[17] probably are true mesotheliomas, but gross and cytologic descriptions were considered inadequate for the purposes of this report. Because our 4 cases illustrate the clinical, morphologic, cytologic, and behavioristic aspects of the other 10 acceptable cases under consideration, they will be presented in detail.

Report of Cases

Case 1

C. K., a white male, 35 years old, was admitted to Temple University Hospital in December, 1939, complaining of pain in the right lower chest of recent onset. He began to show signs of dyspnea and had a cough. He was found to have pleural effusion and thoracentesis was done. During his entire stay in the hospital approximately 64,000 cc. of hemorrhagic fluid was removed from the right pleural cavity, thoracentesis being performed weekly. No neoplastic cells were found in this fluid. The patient developed edema of the face and extremities, concomitant with a lowering of serum proteins. On September 28, 1940, tissue taken for biopsy of the pleura was reported as "malignant tumor of pleura." The patient's condition became progressively worse and he expired on October 11, 1940.

Autopsy (A-2028) was performed by Dr. E. E. Aegerter, 4 hours after death. The body was that of a well developed, moderately well nourished white male. There was some puffiness of the face, moderate edema of the hands, and a very definite pitting edema of the feet and lower one-half of the legs. The entire serosal surface of the peritoneal cavity was studded with small plaques of neoplastic tissue, each plaque measuring 1 to 3 mm. in diameter, being firm and white, and fairly discrete. The plaques were seen in both the visceral and parietal peritoneum. The right parietal pleura was immensely thickened throughout, measuring up to 1.5 cm. in thickness. In places the parietal pleura was perfectly smooth, bluish white, and firm, and in other areas the pleura had a bossed appearance. The right pleural cavity contained 1.5 l. of pale sanguineous fluid. All three lobes of the right lung were completely collapsed and appeared grossly to be entirely and solidly infiltrated by neoplastic tissue. The tumor mass infiltrated the parietal pericardium. The bronchi were carefully dissected and found to be uninvolved by a primary lesion. The appearance was that of a tumor infiltrating the lung from the visceral pleura rather than being primary in the lung. The diaphragm was infiltrated and increased by tumor to a thickness of

about 2 cm. The tumor penetrated the peritoneal surface of the diaphragm. The left parietal and visceral pleurae were thickened and adherent to one another by firm, tallow-colored plaques. The left lung weighed 575 gm. and section disclosed multiple, discrete, pale, firm nodules measuring up to 4 mm. in diameter scattered throughout both lobes. Dissection of the bronchi throughout the lung showed no evidence of a primary lesion. The heart was normal except for a double ostium of the right coronary artery and a very moderate amount of fibrosis of the mitral valve leaflets. The serosal surfaces of the liver, spleen, kidneys, pancreas, and adrenals contained plaques of neoplastic tissue measuring up to 3 cm. in diameter. The gastro-intestinal tract, larynx and trachea, bladder and prostate, thyroid and parathyroid showed nothing of gross pathologic significance. The brain was not examined.

Microscopic sections of the tumor masses disclosed varied cytologic appearances. In many areas there was a dense fibrous stroma surrounding and compressing distorted acinar structures whose individual cells were large, polyhedral, and contained abundant pale eosinophilic cytoplasm. The latter cells occasionally formed stalk-like papillary projections into the acinar lumina, and frequently were found loose within the lumina. In other areas the tumor assumed a distinct sarcomatous appearance, being composed of interlacing bands of spindled cells whose nuclei varied in size, shape, and staining quality. These bands were occasionally interrupted by numerous well formed capillary channels.

Diagnosis. Mesothelioma of the right pleura with extension to mediastinum, left pleura, left lung, diaphragm and peritoneal surfaces.

Case 2

E. F. was a white female, 36 years of age, who was admitted to Temple University Hospital on April 20, 1944. She had been well until approximately 8 weeks prior to admission at which time she began to experience "pain in the left chest." She was admitted to another hospital with a diagnosis of pleurisy and was treated by aspiration. She lost considerable weight, became cyanotic, and her condition became progressively worse. She was transferred to Temple University Hospital for diagnosis and treatment.

Physical examination showed dullness to percussion over the left chest and a shift of the mediastinum to the right. Radiologic examination resulted in the diagnosis of pleural effusion on the left side. Numerous attempts at aspiration resulted in obtaining only small amounts of fluid. The sediment from one of these specimens was reported as "carcinoma, probably metastatic, of pleura." The patient died on May 13 of the same year.

Autopsy (A-3630) was performed by Dr. E. E. Aegerter, 3½ hours after death. The body was that of a remarkably emaciated white female weighing approximately 105 lbs. The serosal surfaces of the peritoneal viscera and omentum were studded by minute, discrete, pale masses each

measuring about 1 mm. in diameter. The capsules of the ovaries were greatly thickened by this tumor process. The entire left thorax was filled with a tumor mass weighing 1900 gm., which completely infiltrated and destroyed all lung tissue except for a small area, 6 cm. in diameter, of atelectatic pulmonary tissue at the apex. Laterally, there were several small cyst-like areas between the pleura and the tumor mass, some measuring up to 5 cm. in diameter. The neoplastic tissue was pale and firm. It diffusely infiltrated lung tissue and extended into and through the mediastinal structures to involve the pleura of the medial aspect of the right thorax. The right lung weighed 275 gm. and showed no evidence of tumor involvement except for several small, discrete, pleural plaques measuring up to 3 mm. in diameter. The right lung was atelectatic because of the mediastinal compression by the immense tumor mass in the left thorax. The left leaf of the diaphragm was infiltrated by neoplastic tissue and its peritoneal surface showed a layer of tumor not unlike that seen in the left lung. The liver, spleen, pancreas, adrenals, kidneys, ureters, and bladder showed nothing of gross pathologic significance. The brain was not examined.

Microscopic sections from the tumor disclosed a varied cytologic pattern. In some areas it was composed of acinar and pseudo-tubular structures lined by a single layer of large cuboidal cells which had a marked tendency to form intraluminal papillary projections. The individual cells were occasionally multinucleated and had a large amount of eosinophilic cytoplasm. The nuclei were round or ovoid. In these areas there was a paucity of stroma, the alveolar structures being closely approximated. Other sections demonstrated an angiomatoid and sarcomatous pattern (Fig. 4), well formed capillary vessels being surrounded by interlacing bands of rather uniform small spindled cells with scant cytoplasm. In these areas there was marked cellularity and the nuclei were small and closely packed.

Diagnosis. Mesothelioma of left pleura with extension to mediastinum, right pleura, diaphragm, and peritoneal surfaces.

Case 3

K. D., a white male, 64 years old, was admitted to Temple University Hospital on May 15, 1944, complaining of swelling of the hands and arms and difficulty in breathing. The patient had noticed enlargement of his neck several months previous to admission. A diagnosis of lymphosarcoma was made by his family physician and the patient was treated over the neck and axillary regions by irradiation, with apparent decrease in the enlargement. He soon began to have difficulty in breathing and was referred to Temple University Hospital for "treatment of a lymphoblastoma." Roentgenograms of the chest showed a "circumscribed mediastinal mass with infiltration of the right lung." The upper extremities were very edematous and the patient had great difficulty in breathing. The blood showed anemia but no evidence of leukemia, and

examination of the sternal marrow revealed nothing of significance. Enlarged nodes from the right axilla removed for biopsy showed fatty infiltration, congestion, edema, and a low-grade chronic inflammatory reaction. The patient became worse, showed signs of cerebral anoxia, and died on May 22, 1944.

Autopsy (A-3646) was performed by Dr. E. E. Aegerter 1 hour after death. The body was that of a well nourished white male. There was great enlargement of the neck and marked edema of the hands and arms extending over the anterior chest. The mesentery and the serosal coverings of the abdominal viscera were involved over large areas by plaques of pale, rather firm neoplastic tissue, giving the appearance of "icing." The mesenteric lymph nodes were enlarged and firm. There was a moderate increase in the peritoneal fluid. When the plastron was removed it was found to be adherent to the anterior mediastinum by neoplastic tissue completely obliterating the right pleural space. The tumor involved both parietal and visceral pleura, and infiltrated the parietal pericardium. The large vessels at the base of the heart were surrounded and compressed by tumor, as were the superior vena cava, the right and left innominate veins, and the innominate, common carotid, and the left subclavian arteries. When the veins were opened, thrombi were found in the lumina. The tumor mass was completely adherent to and infiltrated the diaphragm. The left lung weighed 350 gm. and was moderately atelectatic, and there were approximately 800 cc. of clear fluid in the left pleural cavity. The lung itself showed only moderate congestion. The tumor had invaded the right lower lobe, the rest of the right lung being atelectatic. Dissection of the tracheobronchial tree revealed no evidence of a primary focus of tumor. The heart weighed 335 gm. The myocardium was flabby and the coronary vessels exhibited moderate atherosclerosis. The liver, spleen, pancreas, and adrenals were surrounded by neoplastic tissue. The ureters, bladder, prostate, and intestines were grossly normal. The brain showed a moderate increase in the amount of cerebrospinal fluid and the frontal and parietal convolutions showed moderate atrophy. The brain weighed 1125 gm.

Microscopic sections through various portions of the tumor revealed an unusual structure. The pattern in some areas was definitely mesenchymal (Fig. 2), being composed of pleomorphic spindled cells whose nuclei varied in size, shape, and staining quality. Unbalanced mitotic figures were frequent. The cells contained a moderate amount of pink-staining cytoplasm. Many tumor giant cells were noted. This mesenchymal picture blended into an epithelial pattern (Fig. 1) that demonstrated cross and longitudinal sections of pseudo-tubular structures. The latter were lined by a single layer of large polyhedral cells containing

abundant eosinophilic cytoplasm, their nuclei being round or ovoid. Many of these cells were multinucleated. A minimal amount of fibrous stroma separated these pseudo-tubular structures. The mesenteric lymph nodes revealed only inflammatory hyperplasia.

Diagnosis. Mesothelioma of right pleura with mediastinal extension, compression of great vessels, and diaphragmatic penetration with studding of the peritoneum.

Case 4

M. M. was a white male, 57 years old, who was admitted to Temple University Hospital on February 1, 1949. He had been well until September, 1948, when, following a fall, he noticed recurring pain in the right chest. This pain became worse and the patient was admitted for diagnosis and treatment. Roentgenograms of the chest showed a right pleural effusion. Numerous aspirations of bloody fluid were followed by rapid recurrence of the effusion. Neither cytologic examination of the sediment of centrifuged fluid nor tissue culture study yielded a diagnosis. Finally, a needle biopsy of pleura was done and revealed a malignant tumor, interpreted as metastatic. Later, microscopic examinations of pleural fluid showed clumps of similar tumor cells. After alcohol injection into the pleural cavity the patient was submitted to exploratory thoracotomy, at which time an inoperable tumor mass was found in the right pleural cavity. The patient continued to go downhill and expired about 1 week following surgery.

Autopsy (A-5617) was performed by Drs. P. F. Guerin and W. N. Campbell, 2¼ hours after death. The body was that of a well nourished, well developed white male weighing approximately 200 lbs. The heart and mediastinal structures were pushed into the left chest by a large mass encasing the right lung. The apex of the heart touched the left lateral chest. The left lung weighed 450 gm. and it was partially atelectatic. The tumor mass had completely compressed the right lung. The mass was partially necrotic (post-alcohol injection?) and extended to thicken and infiltrate the pericardial sac and to surround and compress the great vessels. The mediastinal nodes were not enlarged and the left pleura and lung showed no evidence of metastasis. The tumor mass was invading, but had not reached, the peritoneal surface of the right diaphragm. The viable neoplastic tissue was soft and grayish. Examination of the bronchi, bronchioles, and lung parenchyma showed no evidence of a primary focus. The heart weighed 505 gm. and showed only moderate hypertrophy and minimal patchy coronary atherosclerosis. The liver and spleen showed a moderate amount of acute passive congestion. The pancreas, adrenals, intestines, kidneys, ureters, bladder, prostate, thyroid, and brain revealed nothing of gross pathologic significance.

Microscopic sections through the viable tumor disclosed a uniform cytologic picture (Fig. 3). Interlacing masses of cells had small spindled or oval nuclei and an almost complete absence of cytoplasm. Occasional

capillaries in some areas gave the process an angiomatoid appearance. There was little evidence of an epithelial component in the sections examined.

Diagnosis. Mesothelioma of right pleura with extension to mediastinum and partial invasion of right diaphragm.

Discussion

Our 4 cases and the additional 10 cases from the literature will be examined collectively. The cytologic appearance varied considerably. A purely mesenchymal pattern was exhibited by only one of the 14 cases studied (our case 4, Fig. 3). Most of this tumor was necrotic at autopsy, presumably due to the injection of alcohol, and the epithelial component usually associated with pleural mesotheliomas may have been destroyed. Four tumors[3,4,11,14] presented only an epithelial component. Of particular note is the fact that of the 14 tumors under discussion, 10 presented both an epithelial component in some areas and a mesenchymal pattern in others. Thus, although an epithelial pattern is most constant, of much more diagnostic import (and hitherto insufficiently stressed) is the fact that pleural mesotheliomas *usually* demonstrate a cyto-architecture that is epithelial in some areas of the tumor and mesenchymal in others. Many of the cases reported and reviewed in the older literature presented this latter appearance.[7,19]

The character of the epithelial component of these tumors is altered by the amount of collagenous material present between the pseudotubular structures. In our case 3 (Fig. 1), in which a relatively small amount of collagen was present, the histopathologic picture closely resembled that of an adenocarcinoma. In our case 1, on the other hand, there was a huge amount of collagenous material interrupted by clefts or spaces which were lined by cuboidal or polyhedral cells. As a consequence the neoplastic nature of this portion of the process was difficult to appreciate.

The mesenchymal component of some of the tumors simulated fibrosarcoma (case 3, Fig. 2). In others the structure was that of a sarcoma composed of small spindled cells (case 4, Fig. 3). In still others the cells were almost completely undifferentiated.[13] The cells resembled lymphoblastic or reticulum cells in the case reported by Klemperer and Tedeschi.[5] Angiomatoid appearances were an inconstant finding (Fig. 4).

The varied structure of pleural mesothelioma can readily be explained on the basis of the embryologic development of the pleura, the latter arising from the epithelial portion of the mesoderm which possesses

multi-potentialities. Much work has been published to support this view[1,2] which well explains the epithelial and mesenchymal features found in neoplasms arising from the pleura.

On cytologic grounds alone the diagnosis of pleural mesothelioma can be made with reasonable certainty if both epithelial and mesenchymal elements are present in a neoplastic process involving the pleura. Extreme caution should be exercised if only one or the other component is present. This is well illustrated by a case recently reviewed. The gross findings were compatible with the diagnosis of pleural mesothelioma. Some sections of the tumor were indistinguishable from our case 3 (Fig. 1). However, other sections revealed a well differentiated adenocarcinoma whose component cells were tall and columnar. This particular tumor was bronchogenic.

The behavior of pleural mesotheliomas seemed to be the same regardless of the cytologic pattern and is well illustrated by the 14 cases under discussion. The diaphragm was invaded in 10 instances (not mentioned in 2 additional reports) and the peritoneum was studded with tumor nodules in 9 cases. The pericardium was infiltrated by tumor in most instances and occasionally the process extended by contiguity to the mediastinal aspects of the opposite pleura. Occasionally, discrete tumor nodules occurred in the opposite lung and were pleural in location. It would seem that they had occurred through serosal seeding. It would be most logical to assume that *the characteristic and usual method of spread is by contiguity and serosal seeding* in much the same manner as the method of metastasis of a pseudomucinous cystadenocarcinoma of the ovary. This has not been stressed previously and should be of differential diagnostic aid. The neoplasm most easily confused with pleural mesothelioma is bronchogenic carcinoma, which is noted for its frequent widespread metastasis via the arterial blood stream, but is certainly not characterized by serosal seeding. In none of these 14 cases of pleural mesotheliomas was there proved evidence of widespread metastasis to parenchymatous organs. There was a 3 mm. nodule deep in the liver parenchyma in one of the cases reported by Saccone and Coblenz[2] and three nodules in the liver (location not stated) in the case reported by Hertzog and Riley.[15] This, of course, is presumptive evidence of vascular dissemination, but since this was evident in only 2 cases, it is obvious that metastasis via the blood stream is a relatively infrequent finding. Occasional cases presented tumor nodules deep within the opposite lung. For these, the question of bronchogenic dissemination may be raised legitimately.

The hilar lymph nodes were said to be involved by tumor in 8 cases,

but for only 3 of these [4,12,16] was microscopic verification affirmed. An additional case had a microscopic deposit of tumor in a periportal lymph node.[5] The retroperitoneal nodes were said to be involved in 2 cases.[11,12] Microscopic verification of grossly involved lymph nodes is essential in order to determine the frequency of lymphatic metastases. This is well illustrated by case 3 of our series in which enlarged axillary nodes were found to be inflammatory. Further, it is possible that many of the mediastinal and retroperitoneal nodes reported to contain metastatic tumor may have become involved by contiguous spread. More and careful work must be done on this problem.

These results, in general, agree with the interpretations of Saccone and Coblenz,[2] and it seems certain that regional lymphatic metastasis and occasional vascular spread may occur. However, because certain of the tumors reviewed presented extension only by contiguity and serosal seeding, and because the most prominent method of spread of all of the tumors was by this means, we do not feel that the situation is as hopeless clinically as it has seemed; for, if early diagnosis can be established, radical extrapleural surgical extirpation with regional lymph node removal may well result in cure. Since these tumors demonstrate rapid growth and because of the obvious hopelessness once the tumor has penetrated the diaphragm and studded the peritoneum, it is urged that if a definitive diagnosis cannot be made by adequate and intensive study, any patient with unexplained pleuritic chest pain and unilateral pleural effusion that persist for 4 weeks should be subjected to thoracotomy. If tumor is found and deemed operable, radical extirpation would seem indicated even though mediastinal lymph node enlargement is found, for 3 of our cases had inflammatory hyperplasia, only, of the mediastinal lymph nodes.

The incidence of proved diagnoses in our own series is 0.11 per cent, or about one case per 900 autopsies. This agrees with Saccone and Coblenz's statistical analysis.[2] However, over the 8-year period during which our 4 cases were collected, 6 additional surgical specimens of pleura have had histopathologic findings compatible with the diagnosis of pleural mesothelioma. These latter were not included because the diagnosis was not confirmed by autopsy, but they suggest that the actual incidence of the tumor is somewhat higher than has been stated.

Summary

This study is based on 4 new cases of pleural mesothelioma, verified by necropsy, and on 10 recently reported cases. Since most of these 14 cases, and many of the cases in the older literature, exhibited a cyto-

architecture that was epithelial in appearance in some areas and mesenchymal in others, it would appear that definitive diagnosis of pleural mesothelioma can be made in the majority of cases on cytologic grounds alone. The characteristic method of spread of pleural mesothelioma was found to be by contiguity and serosal seeding; this should help differentiate the process from bronchogenic carcinoma. Since pleural mesotheliomas grow rapidly, a plea is made for early diagnosis in the hope that early, radical surgical extirpation may result in cure.

Grateful acknowledgment is made to Dr. Edwin S. Gault for his help with the photomicrographs.

REFERENCES

1. Klemperer, P., and Rabin, C. B. Primary neoplasms of the pleura. *Arch. Path.*, 1931, **11**, 385–412.
2. Saccone, A., and Coblenz, A. Endothelioma of the pleura, with report of 2 cases. *Am. J. Clin. Path.*, 1943, **13**, 186–207.
3. Weissman, H. Primary endothelioma of pleura. *Dis. of Chest*, 1946, **12**, 562–570.
4. Coulter, W. W., Jr. Pleural mesothelioma. *M. Rec. & Ann.*, 1946, **40**, 1376–1380.
5. Klemperer, P., and Tedeschi, C. Pleural mesothelioma. *J. Mt. Sinai Hosp.*, 1941–42, **8**, 710–720.
6. Stout, A. P., and Murray, M. R. Localized pleural mesothelioma. *Arch. Path.*, 1942, **34**, 951–964.
7. Zeckwer, I. T. Mesothelioma of the pleura. *Arch. Int. Med.*, 1924, **34**, 191–205.
8. Scheidegger, S. Über die sog. Endotheliome der Pleura. *Ztschr. f. Krebsforsch.*, 1935, **42**, 93–116. Cited by Saccone and Coblenz.[2]
9. Vitkus, J. T. Pleural mesothelioma. *Ohio State M. J.*, 1937, **33**, 1007–1009.
10. Hochberg, L. A., Epstein, I. G., and Pernikoff, M. Endothelioma (mesothelioma) of pleura (presentation of case treated surgically). *Dis. of Chest*, 1947, **13**, 621–626.
11. Cabot case no. 33111. *New England J. Med.*, 1947, **236**, 407–412.
12. Schiff, L. Case of lung tumor with prominent abdominal changes. *Gastroenterology*, 1946, **7**, 355–363.
13. Evans, T. S., Swirsky, M. Y., and Chernoff, H. M. Primary endothelioma of the pleura. *Ann. Int. Med.*, 1946, **24**, 262–272.
14. Piatt, A. D. Primary mesothelioma (endothelioma) of the pleura. *Am. J. Roentgenol.*, 1946, **55**, 173–180.
15. Hertzog, A. J., and Riley, J. Mesothelioma of pleura. *Minnesota Med.*, 1945, **28**, 209.
16. Postoloff, A. V. Mesothelioma of the pleura. *Arch. Path.*, 1944, **37**, 286–289.
17. Doub, H. P., and Jones, H. C. Endothelioma of the pleura. *Radiology*, 1942, **39**, 27–32.
18. Stephens, H. B. Pulmonary Hemorrhage Associated with Endothelioma of the Lung and Pleura; A Report of 2 Cases. In: Medico-Surgical Tributes to Harold Brunn. University of California Press, Berkeley & Los Angeles, 1942, pp. 525–536.
19. MacMahon, H. E., and Mallory, G. K. Fibrosarcoma of the pleura. *Am. J. Path.*, 1928, **4**, 387–393.

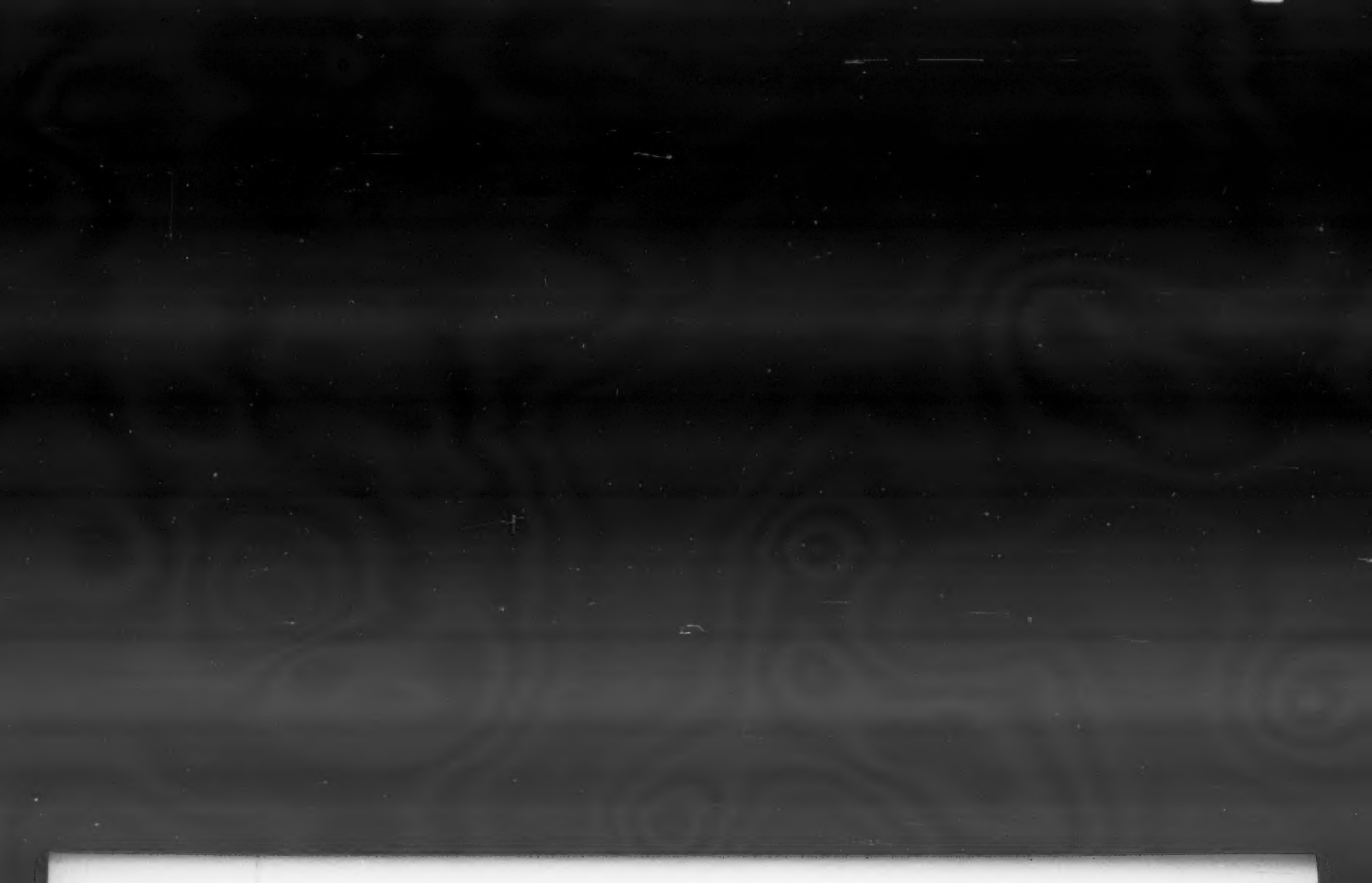
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Case 3. Section showing epithelial pattern. The pseudo-tubular structures are lined by a single layer of cells. The cells are large, polyhedral, and possess abundant eosinophilic cytoplasm. Several are multinucleated. Many cells are seen free within the lumina. Sections from cases 1 and 2 also demonstrated this cellular pattern. Hematoxylin and eosin stain. × 170.

FIG. 2. Case 3. Another area of the tumor shown in Figure 1. This area is definitely mesenchymal in appearance, being composed of pleomorphic, spindled cells. Hematoxylin and eosin stain. × 170.



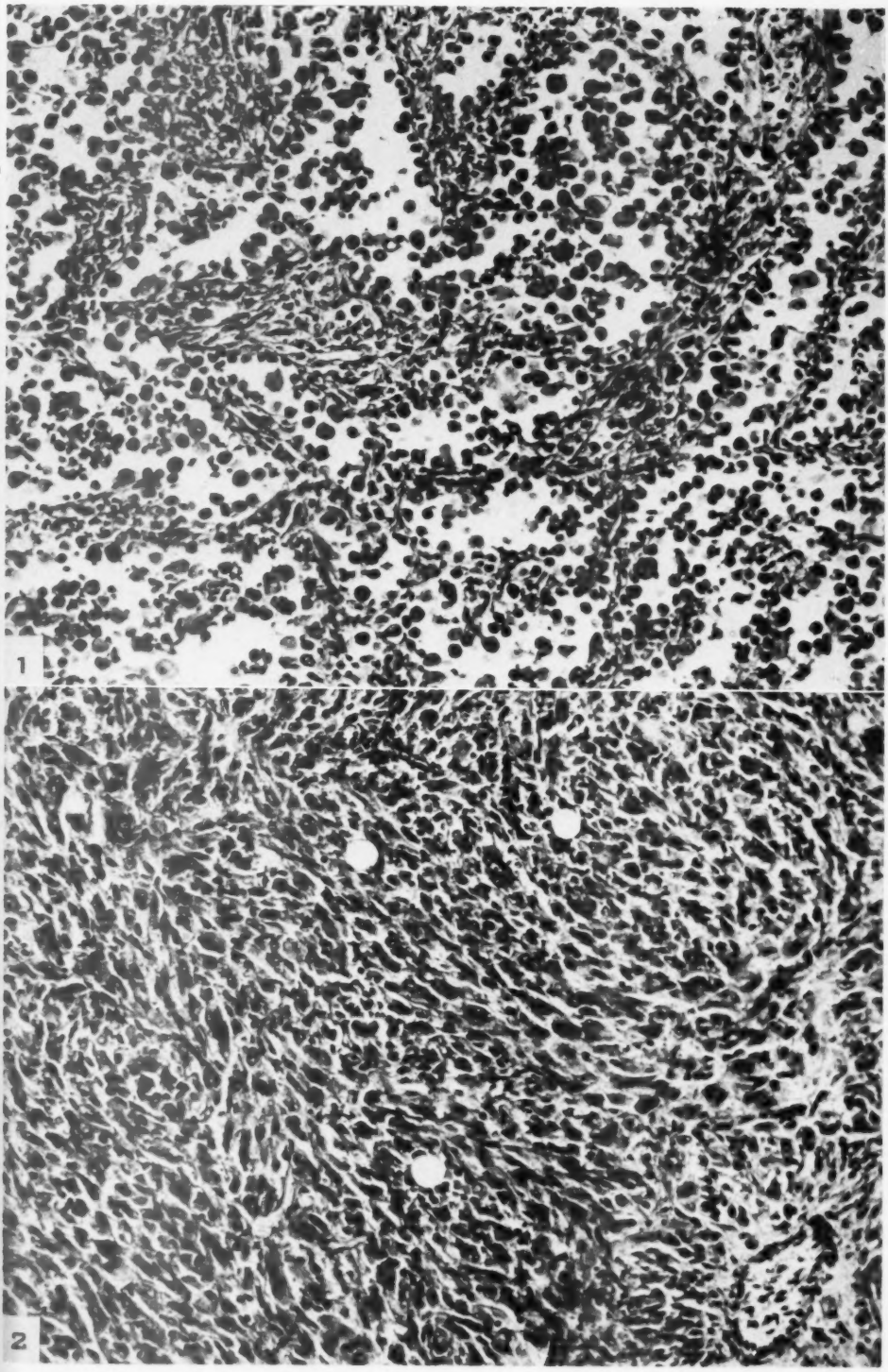
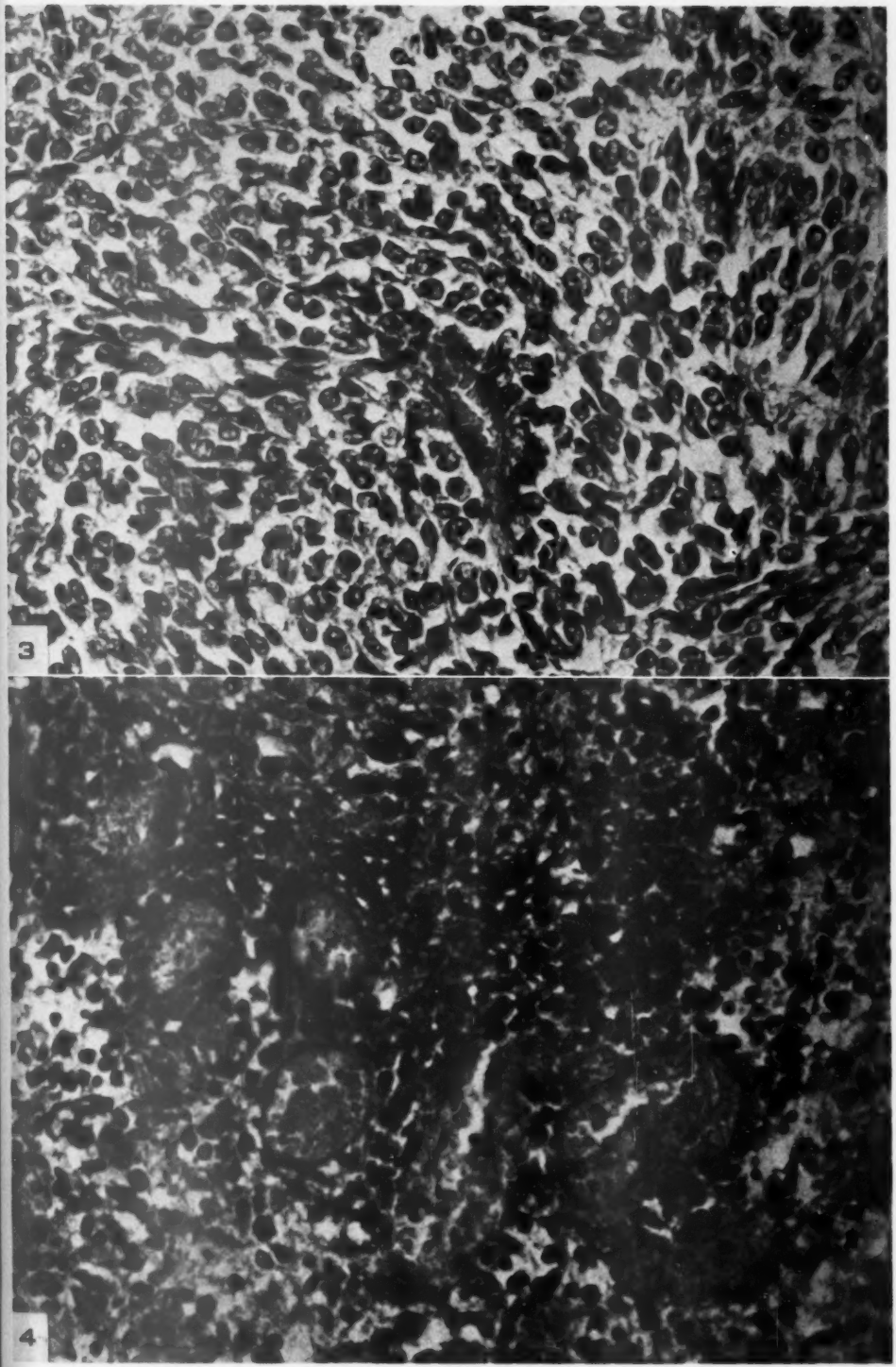

1
2

PLATE 72

FIG. 3. Case 4. Section showing extreme cellularity and mesenchymal appearance. The nuclei are almost naked and are oval or spindled. Some sections of cases 1 and 2 showed a similar pattern. Hematoxylin and eosin stain. × 440.

FIG. 4. Case 2. Section exhibiting angiomatoid features. Areas resembling this were seen also in cases 1 and 4. Hematoxylin and eosin stain. × 440.



Campbell Pleural Mesothelioma

HISTOCHEMICAL STUDIES ON TISSUE ENZYMES
V. A Difficulty in Enzyme Localization in the Acid Range Due To Selective Affinity of Certain Tissues for Lead; Its Dependence on pH *

William Newman, M.D., Elvin A. Kabat, Ph.D., and Abner Wolf, M.D.
(From the Veterans Administration Hospital, Kingsbridge Rd., Bronx, N.Y., the Departments of Neurology, Pathology and Bacteriology, College of Physicians and Surgeons and the Neurological Institute, Presbyterian Hospital, New York, N.Y.)

In the course of a study on the distribution of acid phosphatases, using the Gomori technic,[1] with a variety of phosphate esters and enzyme inhibitors and activators, a previously unrecognized difficulty arose.[1,3] It consisted of occasional, non-specific, but frequently localized staining of various tissues by lead. In some instances, this superficially resembled the histochemical distributions described for acid phosphatases in several organs, notably spleen,[1,3] testis,[1,3] and brain.[3] Lassek[4] described the persistence of "axonal staining" in the brain stem and spinal cord of cat, monkey, and man following a variety of drastic treatments of the tissues, and suggested that the findings with the Gomori technic for acid phosphatase[1] were artifacts, without properly attributing the peculiar effects observed to the staining with lead. It was found in the present study that this non-specific staining by lead varied directly with the pH, occurring infrequently at pH 5.0 and below, increasing in reproducibility and intensity through pH 5.3 to 5.6, and then decreasing in intensity but not in regularity of occurrence at pH 6.0 to 6.8. In addition, at pH 5.6 to 6.8, the distribution patterns in some organs, notably kidney, intestines, testis, epididymis, heart, and brain, looked very similar to those described for alkaline phosphatases,[5-10] and were most striking at about pH 6.0 to 6.4.

The implication that the histochemical localization of phosphatases[1,11] and other esterases,[12,13] in which lead salts are used as the method of visualization, is nothing more than a demonstration of the affinity of various cells and cell components for lead salts in the acid range, led to the present chemical and histochemical re-examination of the validity of the method for localizing acid phosphatases in fixed tissues.[1] In addition, the striking pictures (Figs. 11 to 18) obtained at pH 6.0 to 6.8 with lead alone, stimulated a further evaluation of the technics for alkaline phosphatases too,[5-7] in an effort to show that some metallic

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for opinions expressed or conclusions drawn by the authors.
Received for publication, April 1, 1949.

impregnation might occur in the alkaline range in which calcium is used in place of lead. Danielli[15,16] had previously re-examined the histochemical method for alkaline phosphatases and believed he had shown that some sites of alkaline phosphatase activity also exhibited non-specific affinity for calcium phosphate. It has not been possible to show that calcium in the alkaline range behaves in a manner analogous to lead in the acid range,[10] or to demonstrate any tissue affinity for calcium or calcium phosphate. There is, therefore, no direct evidence of non-specific effects of metallic ions in the alkaline phosphatase technics. It was found, however, that the magnesium ion added as an enzyme activator[8] could, in the absence of calcium, be used to localize sites of alkaline phosphatase activity when the phosphate ion content of the solution became high enough.

The results of this study provide further evidence for the existence of acid and alkaline phosphatases in fixed tissues and show, by chemical experiments to be described below, that the histochemical localization of phosphatases is fundamentally valid provided non-specific effects are adequately considered. The effects of chemical inhibitors and other methods of inhibiting enzyme action, such as heat, may be demonstrated chemically and histochemically using fixed tissues.

Experimental Procedure

Tissues were fixed in cold acetone and prepared as described previously.[3,10] In addition, the effect of routine formalin fixation, which destroys both acid and alkaline phosphatases,[1,3,5,7,10] and 95 per cent alcohol, which inactivates acid phosphatases,[3] were studied.

The histochemical studies of lead staining were done as follows. Adjacent serial sections containing a number of different tissues were incubated in Coplin jars at 38°C. for varying periods of time up to 96 hours, in a series of solutions containing M/16 acetate buffer and M/100 to M/500 lead nitrate, which differed only in the pH to which they had been adjusted. The total volumes, lead concentrations, and subsequent treatment were identical. After incubation, the sections were removed, washed for ½ hour with 6 to 8 changes of distilled water, and placed into a 1 per cent solution of ammonium sulfide for 2 minutes. They were then counterstained with eosin and mounted in balsam. Additional serial sections of the same block of tissue were incubated under identical conditions, except that substrate (sodium-beta-glycerophosphate or glucose-1-phosphate) to give a phosphorus concentration of M/250 was added, and the localization of acid phosphatases was compared with that due to lead impregnation alone. In

like manner, the effect of sodium fluoride in a concentration of M/100, which inactivates acid phosphatases[1,3] but has little or no effect on alkaline phosphatases,[8,10] was studied. The effects of heat (immersing acetone-fixed sections in distilled water at 80°C. for 10 minutes) and of trichloroacetic acid (dipping acetone-fixed sections in 5 per cent trichloroacetic acid for 10 minutes) prior to incubation were tested with and without substrate to observe their effect on acid phosphatases and on the lead staining of tissues. An additional experiment was designed to test the possibility that the products of hydrolysis of various substrates might alter the affinity of different cell components for lead. pH curves with and without the addition of glycerol to the mixtures of lead and M/16 acetate buffer were similar, indicating that the products of hydrolysis had no effect.

Chemical studies of the splitting of substrates by acetone-fixed tissue sections were done as follows. The incubating mixtures, as used in the Gomori technics,[1,3] were prepared with the same substrate concentration, buffer, and total volumes except that lead was omitted in the acid range and calcium in the alkaline range. M/100 magnesium ion was added as activator in the latter instance.[8] Sections cut at 10 μ, from acetone-fixed, paraffin-embedded tissues, were deparaffinized through two changes of xylol, two changes of absolute alcohol, and two changes of 95 per cent alcohol, rinsed with warm tap water, and placed into the mixtures. Just prior to placing the sections in the jars, a 1 cc. aliquot was removed, and the inorganic phosphorus determined according to the Fiske-Subbarow method[17] using a Coleman spectrophotometer at 660 μ with a red filter. A control to measure spontaneous hydrolysis of the substrate was done on a similar mixture to which empty glass slides were added. Phosphorus liberation was measured after incubation for 16, 24, and 48 hours and 1 week for acid phosphatases, and at 2, 6, 24, and 48 hours and 1 week for alkaline phosphatases. In addition, the effect of M/100 sodium fluoride on acid and alkaline phosphatases[3,10] and of M/100 KCN, M/4 glycine, and heat on alkaline phosphatases[10] and on the splitting of phosphates, was evaluated chemically and was found to parallel the action of these substances histochemically when sodium-beta-glycerophosphate was used as the substrate.[10]

In the alkaline range, those jars which contained M/100 magnesium ion to activate alkaline phosphatases[8] showed a more rapid initial liberation of phosphorus than did mixtures without magnesium. However, a maximum level of phosphorus was reached, following which the concentration of phosphorus began to decrease. This could be correlated with the appearance of precipitate on the sections, and when the von Kossa

procedure was done on these sections, a distribution of enzymes similar to that seen with calcium was noted (Table I). The lag is attributable to differences in the solubilities of calcium and magnesium phosphates, longer incubation being required to exceed the higher solubility product of the latter.

TABLE I

Micrograms of Phosphorus Liberated per cc. by Fixed Tissue Sections at pH 9.2; Effect of Magnesium Ion Added as Activator

Hours	0	2	6	24	48	144
Empty glass slides: substrate without added magnesium ion	0.2	0.2	0.2	0.3	0.3	1.1
Empty glass slides: substrate plus M/100 magnesium ion added	0.2	0.2	0.2	0.3	0.2	2.5
Ten fixed tissue sections: substrate without added magnesium ion	0.2	8.5	12.7	47.3	84.5	139.8
Ten fixed tissue sections: substrate plus M/100 magnesium ion added	0.2	15.5	38.2	12.7	11.7	17.4

RESULTS

The histochemical studies indicate that staining by lead is a variable and capricious phenomenon below pH 5.3. It occurred occasionally at pH 4.7, the pH optimum of acid phosphatases,[1,3] and this explained why it was not recognized in earlier studies.[1,3] When staining with lead occurred (in the absence of substrate), it frequently differed from that seen when substrate was added. Thus, in the spleen at pH 4.7 the lead effect (when it was seen at all) was noted only in occasional nuclei in the red pulp, smooth muscle, in trabeculae, and in blood vessels, with increasing numbers of nuclei being affected as the pH increased (Figs. 1 to 3), and there was no cytoplasmic staining, whereas the distribution of acid phosphatases in the spleen at pH 4.7 included nuclei and cytoplasm in the red pulp, nuclei of lymphocytes in the malpighian corpuscle heavily and their cytoplasm lightly, nuclei and cytoplasm of blood vessels, smooth muscle nuclei and occasionally fibers in trabeculae. In the intestines (Fig. 4), the lead effect was seen most frequently in nuclei immediately adjacent to the lumen, only rarely extending to the depths of the glands, and there was no cytoplasmic staining. It frequently was associated with a dense impregnation of the cuticular borders and surface interfaces; lamina propria, muscularis, and nerve plexuses were only occasionally impregnated at pH 4.7. In the presence of substrate, nuclei and cytoplasm of epithelium throughout the gland stained; the

cuticular border was rarely impregnated, and connective tissue nuclei in the lamina propria, smooth muscle nuclei and fibers, and nerve plexuses in the muscularis stained intensely (Fig. 5). In the adrenal, the effect of lead was erratic, showing staining of occasional nuclei in the cortex, the medulla being unstained. In the presence of substrate, the nuclei of both cortical and medullary cells stained deeply, while the cytoplasm of medullary elements was more deeply stained than that of the cortical cells.

There was no attempt to cover completely all organs and tissues in various species, but a lead effect was noted in the following organs at pH 5.3 to 6.0: spleen, heart, liver, small intestine, kidney, lymph node, testis, rete testis, lung, tongue, peripheral nerves, nerves in viscera, stomach, epididymis, pancreas, and adrenal in the rat; brain (Fig. 6) and blood vessels in the rabbit; brain (Fig. 7), thyroid, and white blood cells in man; and brain, testis, epididymis, small intestine, adrenal, lung, and heart in the guinea-pig.

The dependence of the lead effect on pH is illustrated in Table II. The sections containing these organs of the rat were incubated for 24 hours at 38°C. in a lead concentration of M/100.

TABLE II

Dependence of Lead Effect on pH

pH	Spleen	Heart	Liver	Small intestine	Kidney	Lymph node	Testis	Rete testis	Lung
4.5	0	0	0	0	0	0	0	0	0
4.7	+	0	0	0	0	0	0	0	0
5.0	++	+	+	0	Section absent	0	0	0	0
5.3	++	+	+	0	+	0	++	0	+
5.6	++++	++	+++	++	++++	+	+++	+++	+++
6.0	+++	+	+	++	+++	++	+++	+++	+++

0 = No staining. +++ = Moderate staining.
+ = Very light staining. ++++ = Intense staining.
++ = Light staining.

The lead effect was uninfluenced by M/100 sodium fluoride (Fig. 8) which was shown chemically and histochemically to inactivate acid phosphatases (Figs. 9 and 10), so that any staining in the absence of substrate, or any persistence of staining in the presence of substrate plus M/100 sodium fluoride, was due to lead. It was unaffected by formalin and 95 per cent alcohol fixation, both of which inhibit acid phosphatases.[1,3]

Another difference between lead effects and localization as a result of

enzymatic action is the time in which they appear. The staining in the presence of substrate using tissues fixed in acetone and embedded in paraffin was not seen when tissues were incubated for less than 6 to 8 hours. It increased in intensity of deposition following a usual curve of enzymatic activity, and fine histologic detail was lost when tissues were incubated at pH 4.7 for periods above 24 hours (Fig. 9). The optimal time of staining varied somewhat with individual tissue blocks, depending on the care with which they had been prepared. Staining with lead, on the other hand, was noted in as short a period as 15 minutes. The degree of impregnation also increased and more structures stained as the time of incubation was lengthened, maximal staining occurring in 5 to 24 hours. Varying the lead concentration from M/100 to M/500 had no effect.

The effects of staining by lead in the absence of substrate and as a result of enzymatic action in the presence of substrate were similar in two respects. Both were decreased or completely prevented by heating sections at 80°C. in distilled water for 10 minutes, or by dipping sections in 5 per cent trichloroacetic acid for 10 minutes, prior to incubation. In this connection, it should be remembered that the technic devised by Gomori[1] will also reveal deposits of preformed calcium phosphate-calcium carbonate, such as occur in the contents of the intestine or in the developing cartilage. They are relatively unaffected by heat and sodium fluoride, but are soluble in trichloroacetic acid, thus permitting differentiation from both lead effects and enzymatic staining.

As the pH increased toward the alkaline side in the range of pH 6.0 to 6.8, the regions of tissue impregnation by lead salts (in the absence of substrate) became similar, except for less frequent cytoplasmic staining, to those seen for alkaline phosphatases when sodium-beta-glycerophosphate was used as substrate[10] (*Cf.* Figs. 11, 13, 15, and 17 without substrate, with Figs. 12, 14, 16, and 18 with substrate). In the presence of substrate, the effect of acid phosphatases acting away from their pH optimum, plus the non-specific effects of lead, might be misinterpreted as being due to an admixture of acid and alkaline phosphatases.[11]

The chemical experiments are extremely useful in testing for the presence or absence of enzymes in fixed tissues. Stafford and Atkinson[18] have shown that there is a decrease in enzymatic activity in every step through the final embedding of tissues in paraffin, acid phosphatases being more labile than alkaline phosphatases.[8,18] The present method allows the processed sections to be tested for enzymatic activity and

permits evaluation of the effects of various inhibitors and activators on the fixed tissue enzymes. They also indicate that the spontaneous hydrolysis of glycerophosphate and muscle adenylic acid is negligible for periods up to 1 week.[10,11]

Discussion

The relationship of the adsorption of cations to staining procedures has been summarized by Dempsey and Wislocki.[19] McCalla[20-22] and McCalla and Clark,[23] working with viable and killed bacterial cells, were able to show that many cations are adsorbed onto the bacterial cell. Among these are cobalt, lead, mercury, and silver. It has been shown previously that cobalt may be adsorbed on nuclei and hence give a false picture in the localization of alkaline phosphatases.[5,10] In this connection, the effect of cobalt salts in localizing sites of choline esterase activity,[14] particularly in nuclei, should be reinvestigated from the standpoint of non-enzymatic metallic impregnation by cobalt. The findings with lead add another and more serious hazard to the histochemical localization of enzymes.

The staining of nuclei by lead indicates that it combines with some nuclear constituent, possibly nucleoprotein. It is of interest that only certain nuclei in a given tissue stain with lead, although the nuclei which stain cannot be distinguished morphologically from those which do not. Similar variations have been noted in other structures such as axons. The possibility of utilizing lead impregnation of tissues as a staining method should be investigated.[4]

The present observations suggest that some of the discrepancies in the literature on acid phosphatase may be cleared up by relating them to lead effects. The histochemical demonstration of acid phosphatases following fixation[24] may be a non-specific effect due to lead,[4] and the reported pH optimum for acid phosphatases at pH 5.3[25] is probably due to a combination of enzyme action plus lead impregnation.

These findings do not support Gomori's statement that lead impregnation of tissues is most marked below pH 5.3.[13] Since the pH optimum of Gomori's "phosphamidase"[13] is in the range of pH 5.4 to 5.8, the same range in which maximum staining with lead is shown to occur in the present study, it is probable that part or even all of his staining may be due to lead. It would be of importance in this connection to establish chemically that *p*-chloranilidophosphonic acid, the substrate used by Gomori to demonstrate phosphamidase, can be split by fixed tissues.

Similarly, the pictures (Figs. 11, 13, 15, and 17) obtained without substrate and due entirely to lead in the range of pH 6.0 to 6.8 suggest

that Gomori's interpretation of the staining at pH 7.0 as due to admixtures of pH 5.0 and pH 9.0 phosphatases[11] can be explained equally well by lead effects plus acid phosphatases acting away from their pH optimum, or indeed by lead effects alone. This same criticism applies to papers by Dempsey, Deane, and Wislocki[26-28] who have described the histochemical localizations of what they believe to be a group of phosphatases having their pH optima near pH 7.0. The basis for their separation of the phosphatases by substituting phosphate esters other than glycerophosphate has been criticized previously.[10,11]

The chemical studies support the validity of the technic for acid and alkaline phosphatases[1,5-7] in so far as they demonstrate that enzyme is present in fixed tissues. Objections of another character have been raised. Bartelmez and Bensley[29] were concerned with the factor of diffusion in the Gomori technic for acid phosphatases,[1] and they did not believe that the sites of precipitate revealed by the method are necessarily the sites at which enzymatic action is occurring. The papers of Danielli[16] and Baker and Sanders[30] on the rôle of cytochemistry are of particular importance in this connection.

Although the non-specific deposition of lead salts offers a serious handicap for enzyme localization in the pH range of 5.3 to 6.8, the present study suggests that the Gomori technic for acid phosphatases done at pH 4.7, using sodium-beta-glycerophosphate as substrate, and with controls of adjacent sections in a substrate mixture containing M/100 sodium fluoride and with no substrate, may offer valuable information when applied to a wide variety of pathologic tissues if the occasional lead effects at pH 4.7 are subtracted from the total picture due to enzymatic action.

Summary

Staining of tissues by lead in the absence of substrate creates a formidable difficulty in the histochemical localization of enzymes in the range of pH 5.3 to 7.0.

This effect occurs occasionally and variably at pH 4.7, the pH optimum for acid phosphatases, and is unaffected by M/100 sodium fluoride, formalin fixation, and 95 per cent alcohol fixation, all of which inhibit acid phosphatases.

There are stated differences in localization, intensity, and time of occurrence of the "lead effect" without substrate as compared with enzyme staining with substrate.

The effect of lead is relatively slight but erratic at pH 4.7 or below, increasing to maximum intensity and reproducibility at pH 5.6. At

pH 6.0 to 6.8, it decreases somewhat in intensity but not in regularity of occurrence.

At pH 5.6 and above, the distribution pattern obtained with lead appears similar in many organs to that described for alkaline phosphatases, but there is as yet no evidence to suggest that any artifact due to metallic impregnation occurs in the alkaline range.

The existence of acid and alkaline phosphatases in fixed tissues was demonstrated by following the liberation of phosphate chemically. The effects of inhibitors could also be tested.

No significant degree of spontaneous hydrolysis of sodium-beta-glycerophosphate was noted after 1 week in the pH ranges at which the Gomori technics are applied.

Alkaline phosphatases could be localized by precipitation of magnesium phosphate in the absence of calcium. There was an initial lag attributable to differences in the solubilities of calcium and magnesium phosphates.

The present study further establishes the fundamental validity of the Gomori technics for acid and alkaline phosphatases if adequate controls are maintained and the effect of lead impregnation controlled in the acid range.

We wish to thank Mr. Sidney Shapiro, Medical Illustration Division, Veterans Administration Hospital, Kingsbridge, for the photomicrographs and plate mountings, and Mrs. Frances B. Spiegel and Mrs. Lorraine O. Brotman for their technical assistance.

REFERENCES

1. Gomori, G. Distribution of acid phosphatase in the tissues under normal and under pathological conditions. *Arch. Path.*, 1941, **32**, 189–199.
2. Reference withdrawn by the authors.
3. Wolf, A., Kabat, E. A., and Newman, W. Histochemical studies on tissue enzymes. III. A study of the distributions of acid phosphatases with special reference to the nervous system. *Am. J. Path.*, 1943, **19**, 423–439.
4. Lassek, A. M. The stability of so-called axonal acid phosphatase as determined by experiments in its "stainability." *Stain Technol.*, 1947, **22**, 133–138.
5. Gomori, G. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exper. Biol. & Med.*, 1939, **42**, 23–26.
6. Takamatsu, H. Histologische und biochemische Studien über die Phosphatase (I. Mitteilung). Histochemische Untersuchungsmethodik der Phosphatase und deren Verteilung in verschiedenen Organen und Geweben. *Tr. Soc. path. jap.*, 1939, **29**, 492–498.
7. Gomori, G. The distribution of phosphatase in normal organs and tissues. *J. Cell. & Comp. Physiol.*, 1941, **17**, 71–83.
8. Kabat, E. A., and Furth, J. A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. *Am. J. Path.*, 1941, **17**, 303–318.

9. Bourne, G. The distribution of alkaline phosphatase in various tissues. *Quart. J. Exper. Physiol.*, 1943–44, **32**, 1–19.
10. Newman, W., Feigin, I., Wolf, A., and Kabat, E. A. Histochemical studies on tissue enzymes. IV. Distribution of some enzyme systems which liberate phosphate at pH 9.2 as determined with various substrates and inhibitors; demonstration of three groups of enzymes. *Am. J. Path.*, 1950, **26**, 257–305.
11. Gomori, G. Histochemical specificity of phosphatases. *Proc. Soc. Exper. Biol. & Med.*, 1949, **70**, 7–11.
12. Gomori, G. The microtechnical demonstration of sites of lipase activity. *Proc. Soc. Exper. Biol. & Med.*, 1945, **58**, 362–364.
13. Gomori, G. Histochemical demonstration of sites of phosphamidase activity. *Proc. Soc. Exper. Biol. & Med.*, 1948, **69**, 407–409.
14. Gomori, G. Histochemical demonstration of sites of choline esterase activity. *Proc. Soc. Exper. Biol. & Med.*, 1948, **68**, 354–358.
15. Danielli, J. F. A critical study of techniques for determining the cytological position of alkaline phosphatase. *J. Exper. Biol.*, 1945–46, **22**, 110–117.
16. Danielli, J. F. Establishment of cytochemical techniques. *Nature, London*, 1946, **157**, 755–757.
17. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. *J. Biol. Chem.*, 1925, **66**, 375–400.
18. Stafford, R. O., and Atkinson, W. B. Effect of acetone and alcohol fixation and paraffin embedding on activity of acid and alkaline phosphatases in rat tissues. *Science*, 1948, **107**, 279–281.
19. Dempsey, E. W., and Wislocki, G. B. Histochemical contributions to physiology. *Physiol. Rev.*, 1946, **26**, 1–27.
20. McCalla, T. M. Cation adsorption by bacteria. *J. Bact.*, 1940, **40**, 23–32.
21. McCalla, T. M. The adsorption of H^+ by bacteria as measured by the glass electrode. *J. Bact.*, 1941, **41**, 775–784.
22. McCalla, T. M. Reaction of certain stains with bacteria. *Stain Technol.*, 1941, **16**, 27–32.
23. McCalla, T. M., and Clark, F. E. Dye adsorption by bacteria at varying H-ion concentrations. *Stain Technol.*, 1941, **16**, 95–100.
24. Emmel, V. M. The intracellular distribution of alkaline phosphatase activity following various methods of histologic fixation. *Anat. Rec.*, 1946, **95**, 159–175.
25. Noback, C. R., and Montagna, W. Histochemical studies of the basophilia, lipase and phosphatases in the mammalian pancreas and salivary glands. *Am. J. Anat.*, 1947, **81**, 343–367.
26. Dempsey, E. W., and Deane, H. W. The cytological localization, substrate specificity, and pH optima of phosphatases in the duodenum of the mouse. *J. Cell. & Comp. Physiol.*, 1946, **27**, 159–179.
27. Deane, H. W. A cytochemical survey of phosphatases in mammalian liver, pancreas and salivary glands. *Am. J. Anat.*, 1947, **80**, 321–359.
28. Dempsey, E. W., and Wislocki, G. B. Further observations on the distribution of phosphatases in mammalian placentas. *Am. J. Anat.*, 1947, **80**, 1–33.
29. Bartelmez, G. W., and Bensley, S. H. "Acid phosphatase" reactions in peripheral nerves. *Science*, 1947, **106**, 639–641.
30. Baker, J. R., and Sanders, F. K. Establishment of cytochemical techniques. *Nature, London*, 1946, **158**, 129.

[*Illustrations follow*]

DESCRIPTION OF PLATES

All sections were counterstained with eosin. In no instance was a nuclear stain used. Photographs were taken under identical conditions, with a Wratten B no. 58 green filter and Eastman Kodak Panatomic X film. All magnifications were × 120. Within any series, *i.e.* Figures 1–3, 8–10, the technics of exposure and development were identical.

PLATE 73

Rat spleen; no substrate, lead concentration M/100, 24 hours' incubation.

FIG. 1. pH 4.7

FIG. 2. pH 5.3

FIG. 3. pH 5.6

Increasing staining of nuclei in the red pulp with increasing pH. There is no cytoplasmic staining in the red pulp, and the malpighian corpuscles are unstained, unlike the usual distribution pattern seen for acid phosphatases.

FIG. 4. Rat, small intestine; no substrate, lead concentration M/100, 48 hours' incubation at pH 5.3.

FIG. 5. Rat, small intestine; substrate sodium-beta-glycerophosphate, lead concentration M/100, 15 hours' incubation at pH 4.7.

In Figure 4, only occasional nuclei are "stained." There is no cytoplasmic staining. Cuticular borders and interfaces are impregnated. In Figure 5, when sodium-beta-glycerophosphate is used as substrate, nuclei and cytoplasm of epithelium in the entire depth of the gland, nuclei and cytoplasm of connective tissue cells, and fibers in the lamina propria, nuclei and smooth muscle fibers in the muscularis, and nerve plexuses show intense staining and are typical of the distribution of acid phosphatases in this organ.

FIG. 6. Rabbit, medulla; no substrate, lead concentration M/100, 48 hours' incubation at pH 5.3, in the presence of M/100 sodium fluoride.

Axons stand out sharply and distinctly. Nerve cells are unstained in this instance.

FIG. 7. Human brain; no substrate, lead concentration M/100, 48 hours' incubation at pH 5.6.

Nuclei of nerve cells and glia stand out sharply. There is no cytoplasmic staining, which is always a striking feature of acid phosphatases in the central nervous system.

Guinea-pig brain and ganglion.

FIG. 8. No substrate, lead concentration M/100, 46 hours' incubation at pH 4.7, plus M/100 sodium fluoride.

FIG. 9. Substrate sodium-beta-glycerophosphate, lead concentration M/100, 46 hours' incubation at pH 4.7.

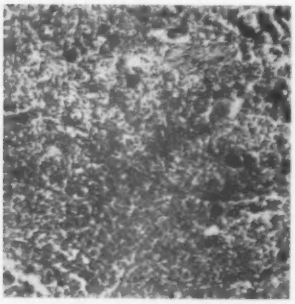
FIG. 10. Like section illustrated in Figure 9 plus M/100 sodium fluoride.

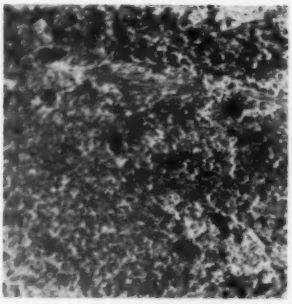
In Figure 8, there is intense staining of ganglion cells and axons. In Figure 9, the intensity of the reaction (staining of nucleus and cytoplasm) makes any histologic differentiation difficult. Figure 10 shows the complete inhibition of enzymatic action by M/100 sodium fluoride. Figure 8 was "stained" in the presence of M/100 sodium fluoride, in the absence of substrate. When "staining" persists in the presence of substrate plus M/100 sodium fluoride at pH 4.7, it is due to lead impregnation of tissues.

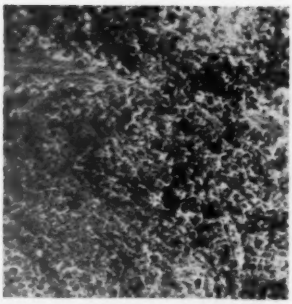
4

6

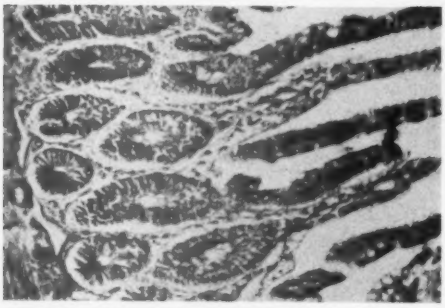
1 2 3

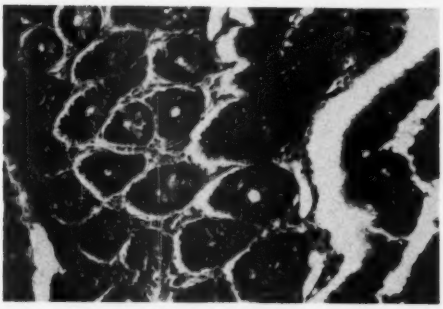






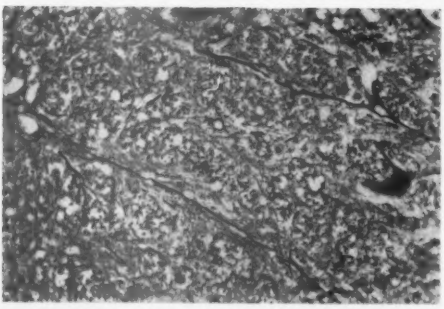
4

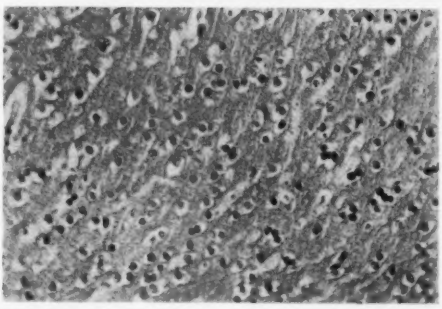




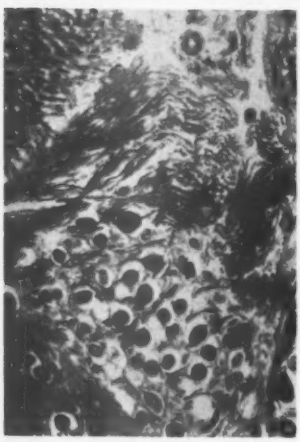
5

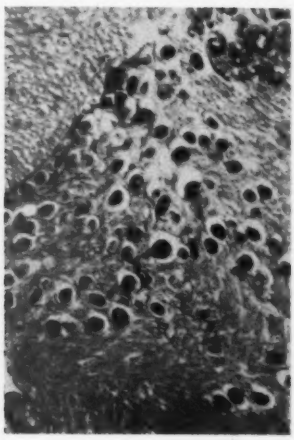
6





7





8 9 10

PLATE 74

FIG. 11. Rat kidney; no substrate, lead concentration M/100, 48 hours' incubation at pH 6.0.

FIG. 12. Rat kidney; substrate, sodium-beta-glycerophosphate, 2 hours' incubation at pH 9.2.

FIG. 13. Guinea-pig heart; no substrate, lead concentration M/100, 48 hours' incubation at pH 6.8.

FIG. 14. Guinea-pig heart; substrate, sodium-beta-glycerophosphate, 2 hours' incubation at pH 9.2.

FIG. 15. Guinea-pig adrenal; no substrate, lead concentration M/100, 48 hours' incubation at pH 6.8.

FIG. 16. Guinea-pig adrenal; substrate, sodium-beta-glycerophosphate, 24 hours' incubation at pH 9.2.

FIG. 17. Guinea-pig brain; no substrate, lead concentration M/100, 48 hours' incubation at pH 6.8.

FIG. 18. Guinea-pig brain; substrate, sodium-beta-glycerophosphate, 2 hours' incubation at pH 9.2.

Figures on the left-hand side of the plate (11, 13, 15, and 17) were "stained" without substrate in M/100 lead. The figures on the right side of the plate (12, 14, 16, and 18) are of the identical organ and species, but are not necessarily from the same animal. They were incubated in sodium-beta-glycerophosphate for the times and at the pH indicated and finished using the von Kossa procedure; they are typical pictures of the distribution of alkaline phosphatases in these organs. Although the distribution patterns are similar, it should be emphasized that to date no evidence of metallic impregnation in the alkaline range has been found.

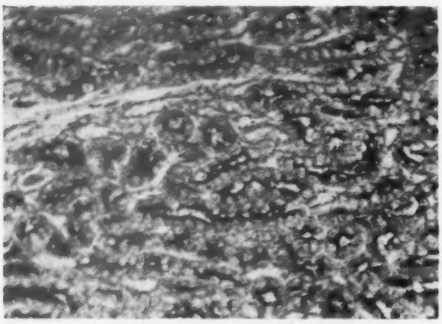
11

13

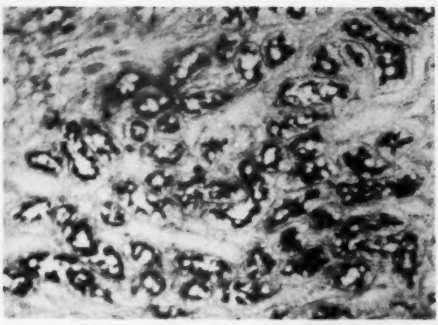
15

17

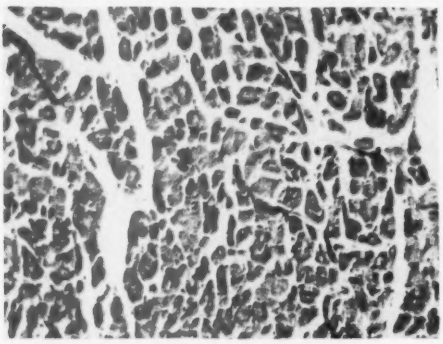
11



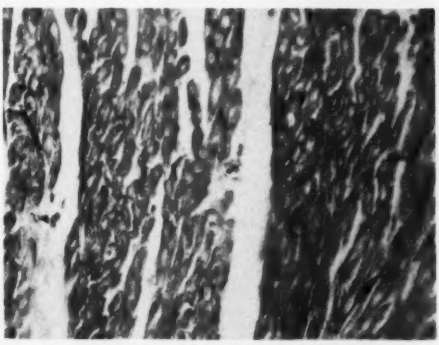
12



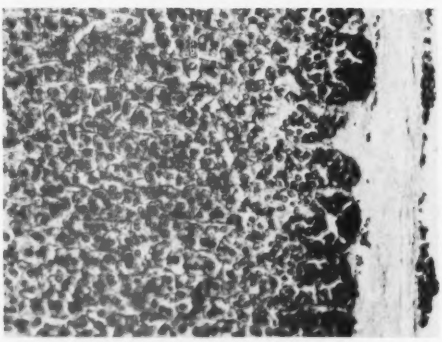
13



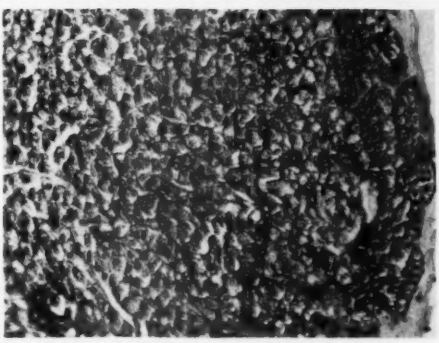
14



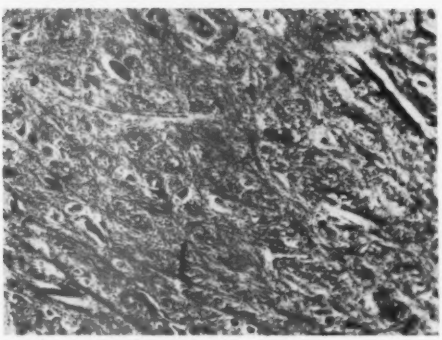
15



16



17



18